THE INHERITANCE AND LINKAGE RELATIONS OF A NEW RECESSIVE SPOTTING IN THE HOUSE MOUSE

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The chief types of spotting thus far described in the house mouse, *Mus musculus*, are ordinary recessive piebald spotting and dominant spotting. Dominant spotting has been shown to be lethal in the homozygous condition because of the anemia associated with it. The purpose of this paper is to describe a new recessive spotting and its linkage relations. Since this new spotting gene is very closely, if not inseparably, linked with the flexed-tail-anemic complex described by HUNT and PERMAR (1928), I shall first review the work that has been done on flexed-tail.

TAIL MUTATIONS IN THE HOUSE MOUSE

In 1910, PLATE described a kinky-tail mutation which appeared in the offspring of four albino mice bought from a fancier in Berlin. From somewhat meagre data he concluded that its inheritance did not follow Mendelian laws. His observations, however, suggest a possible linkage between kinky-tail and pink-eye, and the tail abnormality appeared more often among females than males. He does not mention any anemia associated with the kinky-tail character, although it is possible that it was present but overlooked.

Somewhat later BLANK (1916) made an extensive study of the histology of PLATE's kinky-tail material. He concluded that flexure of the tail is caused by a one-sided fusion of two neighboring tail vertebræ, bringing about an angular arrangement of these joints. He found that the degree of fusion varied greatly and that in extreme cases the whole cross diameter was fused. Bending was caused by a fusion on one side accompanied by continued growth of the epiphyseal groove on the other. The epiphysis on the fused side was broken through or dislodged toward the other side.

HUNT and PERMAR (1928) have described a similar mutation which they have called flexed-tail. This mutation arose in an albino stock secured originally from the BUSSEY INSTITUTION. They found that the character was recessive but gave a ratio of 6.9 normals to 1.0 flexed-tail in the F_2. In a recent publication HUNT, MIXTER, and PERMAR (1933) obtained 40.98 ± 1.12 percent of flexed-tail mice in a backcross. Thinking that their failure to obtain the expected 50 percent of flexed animals might be due to a high postnatal death rate in this class, they tried using only those litters at the final classification (21–28 days) in which no deaths had occurred since birth. The use of these litters increased the percentage of flexed-tail

animals to 43.97 ± 1.44 percent. In an effort to do away with both a differential prenatal and postnatal death rate, undepleted litters of 7 or more animals were used. In these litters, the percentage of flexed-tail mice was 48.30 ± 1.72, which is fairly close to the expected 50 percent. They used the same method on their F_2 data, and were able to increase the percentage of flexed mice from $16.17 \pm .68$ (all litters) to 18.66 ± 1.08 percent (undepleted litters of 7 or more). Even then, however, the deficiency of flexed-tail animals is over 6 percent (25 percent expected). Further on I shall present evidence to show that this apparent deficiency is due partly to normal overlaps.

HUNT, MIXTER, and PERMAR also mated together animals with the same type of flexed-tail to see if the various shapes and forms of the tail were controlled by genetic factors. They were forced to give this up because of sterility which resulted from inbreeding, but found a slight significant correlation between the tail form of parents and offspring as far as they went.

HUNT also noticed that flexed-tail mice are definitely anemic at birth, being lighter in color than their normal-tailed sibs. MIXTER and HUNT (1933) determined the number of erythrocytes and leucocytes, as well as the hemoglobin percentage, in flexed-tail mice and their normal sibs from backcrosses. The normal sibs, of course, would be heterozygous for flexed-tail. A homozygous normal line was also used for an additional control. Unfortunately, the animals used were classified on the basis of flexed-tail rather than upon their anemic appearance. According to data presented in this paper, the flexed-tail animals used were undoubtedly anemic, but the heterozygous control line may have contained a large number of anemics which were genotypically flexed but phenotypically normal-tail. Nevertheless, the comparison between the flexed-tail animals and those of the normal homozygous control line is undoubtedly valid. It was found that the flexed animals were, on the average, deficient at birth in hemoglobin and erythrocytes but became normal at about two weeks of age.

R. J. KAMENOFF, of COLUMBIA UNIVERSITY, gives me permission to state that in an embryological study, as yet unpublished, he has found that flexed-tail mice exhibit anemia as early in embryological development as the 14th day. In a study of the tail, he finds that the fusion between vertebræ in the flexed animals may be unilateral or more or less bilateral, the latter forming the stiff, straight type of joint found in some animals. In both the normals and flexed the mesenchyme differentiates into early or procartilage in the region of the centrum and the intervertebral disc. In normals, this procartilage differentiates into fibers in the intervertebral area and to cartilage in the centrum, while in the flexes, at the point of flexure, it forms cartilage instead of fibers.

HUNT states that occasionally he has obtained flexed-tail mice which are not anemic at birth. In my own crosses, I have observed one such animal, but unfortunately it died. It is very probable that anemia and flexed-tail are caused by two very closely linked genes, the rare flexed non-anemic animals being crossovers. It should be borne in mind, however, that these apparent crossovers may be flexed individuals that recover from the anemia before birth.

DANFORTH (1930) believes that flexed-tail is caused by persistence of the caudal intestine which throws the vertebræ out of alignment.

R. J. KAMENOFF (1932) exhibited slides of specimens obtained in an embryological study of the development of flexed-tail. He says: "This character is expressed as a unilateral fusion between successive tail vertebræ, as seen in the cleared specimens. In the fourteenth day of gestation the mesenchyme of the invertebral anlage fails to form fibrous tissue on one side of the bent notochord. The successive slides show this non-fibrous anlage forming a cartilaginous bridge between the adjoining cartilaginous vertebræ. This bridge's later ossifying prevents sub-epiphyseal growth on that side, thus accentuating the angle and pushing the nucleus pulposus to the lateral position seen in the slide of the adult."

DOBROVOLSKAIA-ZAVADSKAIA (1927a, 1927b, 1928a, 1928b) and DOBROVOLSKAIA-ZAVADSKAIA and KOBOZIEFF (1927a, 1927b, 1928, 1930a, 1930b) have described a dominant lethal mutation in the mouse which results in partial or complete absence of the tail, and in various flexures and constrictions of the tail remnant. In some cases a little coiled persistent filament is all that represents the tail. Certain vertebræ may be missing in the back as well as in the tail. It was shown that mice homozygous for this dominant gene die during embryonic life.

In a recent paper in which symbols for this anomaly are discussed, DOBROVOLSKAIA-ZAVADSKAIA (1933) attributes these tail defects to the action of one dominant lethal gene (*T*) in combination with various modifying genes. She states that various tail forms which emerged during breeding experiments in mice with this mutation were not only transmitted from parent to offspring, but could also be isolated by selection into separate lines.

In my own crosses, I have found that a very close fit to a 1:1 ratio is obtained when short-tailed mice are mated with normals, but the tail anomaly may vary tremendously in its expression, even among the offspring of a single cross.

An embryological study may result in a better understanding of the gene or genes causing this tail abnormality. I am working on the linkage relations of this character and hope to report on it in the near future.

Another occurrence of interest is the appearance of a dominant muta-

tion for flexed-tail noticed by Dr. KEELER in the mouse colony of the BUSSEY INSTITUTION. This type of flexed-tail is very similar in its phenotypic expression to recessive flexed, except that no anemia is associated with it. Some individuals show a shortening or thickening of the tail and look exactly like mice carrying the gene of brachyury. Preliminary data, however, indicate that this gene does not have a lethal effect when homozygous.

BELLYSPOT, FLEXED-TAIL AND ANEMIA

While trying to isolate various piebald patterns in Dr. HUNT'S laboratory at MICHIGAN STATE COLLEGE, I noticed that many of his flexed-tail

FIGURE 1.—Bellyspot.

mice had a bellyspot about the size of a dime on the abdomen. In segregating populations, this character varies phenotypically from no spot at all (normal overlap) to a large spot covering most of the ventral surface and, in rare cases, extending up the sides in the form of a partial belt. Except for these rare cases in which the spot extends up the sides, bellyspot, as the name signifies, is limited to the ventral region. In this respect, it is similar to the Irish spotting factor in the Norway rat. Mice which are homozygous for bellyspot have white toes and may or may not have a spot on the tip of the tail. A mouse with a typical bellyspot is shown in figure 1.

Two flexed-tail bellyspot males from HUNT'S stock were crossed with females from LITTLE'S inbred black non-spotted strain. Sixty-seven F_1 mice were obtained, all of which showed no spotting (except on the tips of the toes). Bellyspot, therefore, behaves as a recessive. A large F_2 popu-

TABLE 1

F_2 flexed-tail bellyspot×normal self (coupling).

	NORMAL	BELLYSPOT NON-FLEXED	FLEXED NON-SPOT	BELLYSPOT FLEXED	TOTAL
Observed	494	15	1	70	580
Expected 9:3:3:1	326.25	108.75	108.75	36.25	
Dev.	167.75	−93.75	−107.75	33.75	
P.E.	8.06	6.34	6.43	3.93	
Dev./P.E.	20.81	14.76	17.00	8.59	

lation was obtained which is classified in table 1, the results indicating that bellyspot and flexed-tail are closely linked. The parental classes are greatly in excess, the deviations from a 9:3:3:1 ratio being many times greater than the respective probable errors for the four classes. Only 71 flexed animals appeared out of the expected number, 145, and only 85 bellyspot animals were recovered out of the expected number, 145. There are indications that the deficiency of flexed animals is due to normal overlaps rather than to high mortality and that bellyspot likewise gives a high percentage of normal overlaps, that is, fails to appear in the phenotype, though present in the genotype. The fact that 70 of the 71 phenotypically flexed mice also had a bellyspot indicates, in spite of normal overlaps, that these two characters are very closely linked. Applying the formula of FISHER and BALMUKAND (1928), this distribution indicates less than 2 percent of crossing over.

Some of the F_2 double recessives, bellyspot flexed animals, were backcrossed to the F_1 with the results shown in table 2.

TABLE 2

Backcross involving flexed-tail and bellyspot (coupling).

	NORMAL	BELLYSPOT NON-FLEXED	FLEXED NON-SPOT	BELLYSPOT FLEXED	TOTAL
Observed	382	45	9	149	585
Expected 1:1:1:1	146.25	146.25	146.25	146.25	
Dev.	235.75	−101.25	−137.25	2.75	
P.E.	7.06	7.06	7.06	7.06	
Dev./P.E.	33.39	14.34	19.44	.39	

Since this is a backcross, we should expect a 1:1:1:1 ratio, if there were no linkage. The bellyspot flexed class fits very closely to the expected number on the basis of no linkage. That this is merely an effect caused by normal overlapping is shown by the poor fit of the other classes and by the data on normal overlaps which follow. The other parental class is

present in great excess, while the two crossover classes are strikingly deficient. These other three classes indicate a strong linkage between flexed-tail and bellyspot. Other data which have since been obtained throw considerable light on these results. As the classification of the backcross young stands, it indicates a crossover percentage of about 9.

Thirty-five litters, with a total of 187 mice, were obtained by mating double recessives, bellyspot flexed animals, *inter se*. Of these 187 mice, 57 were normal (double overlaps), 26 were bellyspot non-flexed (overlaps for flexed), 33 were flexed non-spot (overlaps for spot), and 71 were bellyspot flexed, like their parents. All these mice were anemic at birth, which is, as I shall show later, sufficient evidence that they are homozygous for

FIGURE 2.—Flexed-tail.

flexed-tail and bellyspot, whether they show them phenotypically or not. We find from these data 30.5 percent double overlaps, 48.1 percent overlaps for bellyspot, and 44.4 percent overlaps for flexed. This proves that both bellyspot and flexed-tail give a high percentage of normal overlaps and indicates that in the F_2 and backcross data some of the individuals in the crossover classes (bellyspot non-flexed and flexed-non-spot) were overlaps and not true crossovers at all. If this is true, the linkage is even closer than the F_2 data indicate. Data from other crosses reveal more fully the relationship between these two characters.

Flexed-tail animals, except for possible rare exceptions, are anemic at birth. HUNT has reported several such exceptions. I have looked carefully for such animals in large F_2 and backcross populations and have found only one, which died soon after birth. If true crossovers do occur between

flexed-tail and anemia, they are very rare indeed. We may use anemia, then, as an index of whether or not a mouse is genotypically flexed-tail.

It has been noticed that bellyspot animals are always anemic. Bellyspot anemic mice were crossed with a non-spotted inbred strain, and the numbers of young indicated in table 3 were obtained.

TABLE 3

F_2 bellyspot anemic×normal self.

	NORMAL		ANEMIC		TOTAL
	SELF	BELLYSPOT	SELF	BELLYSPOT	
Observed	205	0	29	36	270

It will be noticed that there is a good 3:1 ratio of normal to anemic mice (205:65), but that only slightly more than half of the anemics bear a bellyspot. However, none of the normals are bellyspot. Hence there is no indication of crossing over between anemia and bellyspot, but rather a failure of bellyspot to express itself in 44.6 percent of the anemic mice. In other words, 44.6 percent of the anemics are overlaps for bellyspot. This is a close approximation to the percentage obtained when bellyspot animals are mated *inter se* (48.1 percent). That such unspotted (self) animals are genetically bellyspot is shown by evidence presently to be discussed. A close association of this kind between bellyspot and flexed-tail anemia is somewhat analogous to that existing between dominant spotting and its lethal anemia.

Further evidence of the close association of bellyspot, flexed-tail, and anemia is revealed by *inter se* matings of animals from the F_2 supposed crossover class, bellyspot non-flexed. Three pairs of such mice were mated and produced 38 young, all of which were anemic, indicating that the parents were homozygous for anemia. (Animals recorded in tables 1 and 2 were not classified for anemia.) Eighteen of the young were bellyspot, while 20 were non-spot, which gives 53 percent of normal overlaps for bellyspot. Eleven animals were flexed-tail (ten of them also bellyspot). At first thought, this might appear like a 3:1 segregation for flexed-tail, but since flexed-tail animals are almost certain to be anemic, it is logical to conclude that the parents were overlaps for flexed and not true crossovers.

Flexed non-spot mice were also mated *inter se*. All of them were either obtained from the so-called flexed non-spot crossover class in table 2, or resulted from *inter se* matings of that class. Seven such matings, involving seven females and two males, produced 57 young, all of which were anemic. Of the 47 young which survived, 15 were flexed non-spot like the parents, 13 were bellyspot, of which 7 were flexed and 6 non-flexed, and

19 showed neither bellyspot nor flexed-tail. Since all the young were anemic we may safely assume that the parents were also homozygous for anemia. Because of failure in all our crosses to find any bellyspot mice which were not anemic, it seems reasonable to conclude that the 13 bellyspot animals which appeared from these matings were not the result of simple segregation but were produced from parents which were overlaps for spotting (duplex for bellyspot, anemia, and flexed-tail). In this case, then, 30 percent of the young were bellyspot and so genetically homozygous for the bellyspot gene. The percentage of normal overlaps for flexed from this same cross was 53.

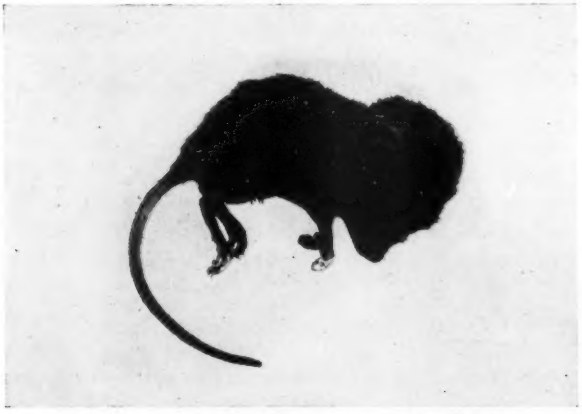

FIGURE 3.—Hydrocephalus.

We may conclude, then, that bellyspot, flexed-tail, and anemia are due to the same gene, or to very closely linked genes, for the following reasons:

1. Flexed-tail animals, in my observation, with one exception, have shown anemia.

2. Bellyspot mice likewise regularly exhibit anemia, or if not observed to be anemic, are shown by subsequent breeding tests to be homozygous for the character.

3. In segregating populations, no bellyspot mice have appeared which were not anemic, and only one non-anemic flexed-tail animal has been observed.

4. *Inter se* matings in the apparent crossover classes, flexed non-spot and bellyspot non-flexed, produced only anemic young.

INTERPRETATION OF F_2 AND BACKCROSS RESULTS (TABLES 1 AND 2) ON THE BASIS OF COMPLETE LINKAGE WITH NORMAL OVERLAPS

We may assume, from data already presented, that there is no crossing over between bellyspot and flexed-tail. If such is the case, the bellyspot non-flexed and flexed non-spot classes in tables 1 and 2 are not crossover classes but are merely overlaps which genotypically belong in the double recessive, bellyspot flexed class. If we add these classes together in table 1, we get a ratio of 494 to 86, which is a very poor fit to the expected 3:1 ratio. Data given later, on mortality among anemic mice, indicate that the poor fit is due to overlaps rather than to differential mortality. Considering flexed-tail alone, we have a ratio of 509 normal to 71 flexed-tail, where we should expect 435 normal to 145 flexed-tail. Approximately 74 of the normals are probably overlaps for flexed, this amounting to 51 percent normal overlaps for flexed. Of these flexed overlaps, 15 are doubtless found in the bellyspot non-flexed class. The remaining 59, we may assume, are to be found in the normal class.

For bellyspot we get a ratio of 495 non-spot to 85 bellyspot, where we should expect a ratio of 435 to 145. Therefore, 60 of the non-spotted class should be overlaps for bellyspot. This would indicate about 41.4 percent of overlaps for bellyspot. Fifty-nine of the 60 overlaps would be in the normal class, and one would be represented by the single flexed non-spot individual. Since, with both flexed-tail and bellyspot, 59 overlaps must be looked for in the normal class, these 59 will be double overlaps. The percentage of double overlaps, then, is about 40.7, and single overlaps for either bellyspot or flexed, 11.0 percent.

In the backcross we should expect a 1:1 ratio of normal to bellyspot flexed, if there were no normal overlaps. The ratio obtained was 382 normal to 203 which phenotypically are duplex for one or both recessive factors. Considering flexed-tail alone, a ratio was obtained of 427 non-flexed to 158 flexed, when theoretically we should expect 292.5 to 292.5. This gives us 134 overlaps for flexed, or 46 percent. Forty-five of them would constitute the bellyspot non-flexed class, and the remaining 89 would be expected to occur in the normal class.

With bellyspot, we get a ratio of 391 non-spot to 194 bellyspot, or a deviation from the expected 1:1 ratio of 98.5. This gives 33.7 percent normal overlaps for bellyspot. Eighty-nine of the 98.5 overlaps would be double overlaps. The double overlaps (overlaps for both flexed and bellyspot) would be 30.4 percent, and there are 18.4 percent single overlaps (for bellyspot or flexed).

Table 4 shows the percentage of double, single, and total overlaps obtained with bellyspot and flexed-tail from different types of crosses. A glance at the F_2 results on bellyspot (crosses 1 and 6) shows that from 40

TABLE 4

Percentage of overlaps obtained from various crosses.

CROSS	DOUBLE	SINGLE		TOTAL		TOTAL NUMBER MICE
		BELLYSPOT	FLEXED	BELLYSPOT	FLEXED	
1. F_2 involving bellyspot and flexed	40.7	0.1	10.3	40.8	51.0	580
2. Backcross, bellyspot and flexed	30.4	3.1	15.4	33.5	45.8	585
3. Bellyspot flexed *inter se*	30.5	17.7	13.9	48.2	44.4	187
4. Bellyspot non-flexed *inter se*				30.8		26
5. Flexed non-spot *inter se*					53.2	47
6. F_2 of bellyspot				44.6		193
Weighted average	34.8	3.8	13.0	39.3	48.0	

to 45 percent bellyspot overlaps may be expected in an F_2 population. The percentage may be expected to vary, depending upon the spotting modifiers present in the strains used. The inbred strains used were LITTLE'S inbred non-agouti black strain in cross 1, and for cross 6, several strains were used. Overlaps of flexed-tail alone varied from 45 to 50 percent. Between three-fourths and four-fifths of all the overlaps obtained were double overlaps involving both bellyspot and flexed-tail.

Since the matings together of bellyspot flexed give 30 percent of double overlaps, we should expect to obtain a like percentage in a backcross population, which is the case if complete linkage is assumed. Since data given later indicate conclusively that mortality among flexed anemic animals is only 10 percent greater than among normals, serious distortion of the ratios by differential mortality is ruled out. Both crossover classes should appear in nearly equal numbers, if there were true crossing over, but in the F_2 they are in the ratio of 15:1, and in the backcross 45:9. Also the double recessive class in a backcross should be nearly equal in number to the normal class, but actually it is less than half as large. In view of the evidence presented, the only satisfactory explanation of the F_2 and backcross ratios is to assume complete or nearly complete linkage of bellyspot and flexed with a high percentage of normal overlaps. Such an assumption is in accord with the complete association of anemia with bellyspot and the probable complete association of anemia with flexed. In the latter case, crossing over, if it occurs, is very rare.

MORTALITY RATES IN CROSSES INVOLVING BELLYSPOT-FLEXED-ANEMIA

Bellyspot-flexed-anemic animals were crossed with an inbred non-spotted dilute brown strain. No spotting occurred in the F_1 mice. An F_2 population consisted of 644 animals and, in the case of the anemics, the

tip of the tail was snipped off soon after birth, so that they could be recognized later. The ratio of normal to anemics at this time is shown in table 5.

TABLE 5

F_2 from anemic×normal, classified at birth.

	NORMAL	ANEMIC	TOTAL
Observed	477	167	644
Expected 3:1	483	161	
Dev.	−6	6; P.E. 7.41; Dev./P.E. 0.84	

Good agreement with a 3:1 ratio is found, the deviation being only 0.84 of the probable error. This shows that the prenatal mortality is no higher among anemics than among normal mice. These same litters were then examined after weaning (20 to 30 days after birth), and the numbers recorded in table 6 were found.

TABLE 6

Same litters as classified in table 5, when observed at 20 to 30 days of age.

	NORMAL	ANEMIC	TOTAL
Observed	430	131	561
Expected 3:1	420.75	140.25	
Dev.	9.25	−9.25; P.E. 6.92, Dev./P.E. 1.34	

The numbers obtained from the second classification still fit a 3:1 ratio fairly well. The Dev./P.E. is 1.43. The odds are only 1.63 to 1 against such a deviation being due to chance. A comparison of the first and second classifications would indicate that meanwhile 47, or 9.85 percent of the normals died, while 36, or 21.56 percent of the anemics died. Although the postnatal mortality is thus twice as high among anemics as among normals, the difference is not great enough to affect the ratios seriously in a population of four or five hundred animals.

Additional F_2 data involving anemia, obtained from other crosses, may be combined with the data recorded in table 5, giving the result shown in table 7. In all cases, the classification for anemia was made at birth. The

TABLE 7

F_2 from anemic×normal, classified at birth.

	NORMAL	ANEMIC	TOTAL
Observed	1240	405	1645
Expected 3:1	1233.75	411.25	
Dev.	6.25	−6.25; P.E. 11.85; Dev./P.E. 0.53	

very close fit to a 3:1 ratio indicates that this type of anemia behaves genetically as a simple Mendelian recessive. This conclusion is supported further by backcross results shown in table 8.

TABLE 8

Backcross involving anemia, classified at birth.

	NORMAL	ANEMIC	TOTAL
Observed	62	56	118
Expected 1:1	59	59	
Dev.	3	−3; P.E. 3.66; Dev./P.E. 0.82	

HUNT, MIXTER, and PERMAR (1933), although they mention the probability of normal overlaps occurring in their backcross and F_2 data, emphasize mainly the high prenatal and postnatal death rate in the flexed-tail classes and endeavor to correct for this by using only large undepleted litters. My results indicate that the prenatal death rate is no higher among flexed than among normal mice, and that the postnatal death rate, although higher in the flexed group, is not enough higher seriously to affect the final count.

The distribution of anemic mice in the F_2 and backcross classes of the data presented by HUNT, MIXTER, and PERMAR indicates the presence of normal overlaps for flexed. In a backcross, they obtain at birth only 3 flexed animals which are not anemic out of a total of 347 flexed-tail mice. If these 3 animals represent crossovers between flexed-tail and anemia, the other (complementary) crossover class to be expected would be normal-tail anemic, and this should be approximately as large as the first crossover class. Even if crossing over does take place, some discrepancy might exist between the two crossover classes because of the difficulty of classifying flexed-tail at birth. We find, however (table 2), that there are 78 normal-tail anemics and 28 with an intermediate or doubtful tail. My data indicate that flexed-tail and anemia are very closely, if not inseparably, linked. All these facts tend to show that the anemics in both the normal-tail and doubtful tail classes are really overlaps for flexed-tail. If we assume that they are, and put them in the flexed-tail class, we find that the percentage of flexed-tail animals at birth is 47 and the percentage of normal overlaps is about 23.4.

HUNT, MIXTER, and PERMAR, by using undepleted litters of 7 or more mice, obtained 18.66 ± 1.08 percent of flexed in F_2, where 25 percent would be expected. Thus the flexed class shows a shortage of over 6 percent, even when large undepleted litters are used. If we take the anemics in the normal-tail class and half of the mice in the classes in which the condition of

the blood was uncertain and include them in the flexed class, the percentage of flexed animals is raised to 22.2 percent. Even then the ratio differs significantly from the expected 3:1 proportion. If we include all those animals whose blood condition was uncertain in the flexed class, the percentage is raised to 23.2. The ratio deviates, then, from a 3:1 ratio by only 25.2 ± 11.24.

In my experiments, no difficulty has been experienced in detecting anemic segregates, provided the litters were classified the day they were born. Also, I found no evidence of a higher prenatal death rate among flexed mice than among normals, and the postnatal death rate was only 10 percent higher among anemics (flexed) than among normals. My matings of flexed × flexed gave many normal overlaps, while HUNT obtained scarcely any. Possibly our strains differ in the number of factors present that modify the action of the flexed-tail gene. In view of my results showing the close relationship between flexed-tail and anemia, it is logical to conclude that part of the shortage of flexed-tail mice in HUNT'S tables is due to normal overlaps. Also, part of the shortage, as he has shown by using large undepleted litters, is due to a higher mortality in the flexed class.

OTHER TYPES OF SPOTTING IN THE HOUSE MOUSE

The discovery by SNELL (1931) that piebald spotting is linked with hairless has greatly simplified the study of new types of spotting in the mouse. Uncertain results, due to difficulty of classification, are usually obtained when two types of spotting enter into the same cross. Now, however, new spottings can be differentiated from piebald by crossing with a self hairless strain. This method is being employed by Dr. C. E. KEELER of the BUSSEY INSTITUTION in the analysis of several new spottings. One of these is *Akhissar spotting* (KEELER 1933b). This type of spotting arose from a male mouse with a small bellyspot captured by KEELER in a grain shop at Akhissar, Turkey. When the Akhissar spotted strain was crossed with a self strain, there were obtained 7 self young and two having white-tipped tails. In the F_2 many selfs appeared, in addition to mice showing all grades of spotting from white-tipped tails to belted. Akhissar spotting was crossed with self hairless, and the F_2 data indicate that it is a form of recessive piebald. This is a very good example of a piebald strain developed in the laboratory by selection of a wild sport.

Bar Harbor bellyspot. LITTLE'S inbred non-agouti chocolate mice frequently exhibit a small spot on the belly. J. M. MURRAY informs me that this type of spotting appears to be genetically different from dominant and piebald spotting. My results show that it is not identical with the bellyspot in my stock.

Mutation to white-bellied agouti. Four mutations to white-bellied agouti

have been reported by LITTLE (1916), and five mutations to black and tan have also been reported, one by PINCUS (1929) and four by SNELL (1931).

In my own crosses one mutation to white-bellied agouti has been encountered as follows. A flexed-tail dark belly agouti animal, which carried non-agouti, was crossed with a black non-agouti from KEELER'S black rodless strain. All the F_1 animals were either black non-agouti or black agouti with dark belly. In this particular case two F_1 males (one was agouti and one non-agouti) were mated in a stock cage with several F_1 females. The females, when pregnant, were isolated. One of the agouti F_1 females had a litter of nine wild-type young, one of which had a white belly. This mutant was mated with a black non-agouti male and produced 7 white belly agouti young and 12 dark belly black non-agouti. All three F_1 animals were tested further, but no more white-bellied young appeared in the F_2. This indicates that only one, or possibly a few, of the germ cells of one of the F_1 animals mutated to the white-bellied condition.

LINKAGE TESTS

Linkage tests were made for the purpose of finding out whether the gene (or closely associated genes) causing bellyspot, flexed, and anemia are linked with any of the other known genes of mice.

Test for linkage between bellyspot-anemia and hairless

Since bellyspot is a recessive spotting, it was deemed important to determine whether or not it is an allele of the piebald gene, also recessive and at times phenotypically similar. But hairless is known to be linked rather closely with piebald, with only about 9 percent of crossing over. Accordingly, if hairless were shown to be independent of anemia, it would follow that piebald also must be independent of anemia, a fact which it would be difficult to establish by a direct cross between anemia and piebald, since it would be impossible to discriminate with certainty between true piebald and bellyspot, both of which would be expected to occur in F_2.

Crosses were made with a hairless strain which was dominant spotted (*Ww*). Since my tests with *Ww* (presented on pages 26 to 30) show that bellyspot is not linked with that gene, I considered it valid to disregard all spotting in this cross, and base my conclusions on the relationship between anemia and hairless. Of course *WW* anemics will be present at birth in F_2, but their early death will take place before the time of classification. Those anemics that live will be of the type associated with bellyspot and flexed-tail. Since piebald is linked with hairless with about 9 percent crossing over, if bellyspot actually were a form of the piebald gene, we should expect in F_2 only about two anemic mice in 64 to be hairless. The cross was made as follows:

P_1 *Ww hrhr FF*×*ww HrHr ff*
F_1 *Ww HrHr Ff spotted*
ww Hrhr Ff self

An F_2 population was obtained by mating the spotted F_1's (*Ww Hrhr Ff*) *inter se*. The results are as follows (disregarding *Ww*).

TABLE 9

F_2 involving hairless and bellyspot anemia (repulsion).

	NORMAL		ANEMIC		TOTAL
	Hr	*hr*	*Hr*	*hr*	
Observed	67	24	16	4	111
Expected 9:3:3:1	62.4	20.8	20.8	7.0	
Dev.	4.6	3.2	−4.8	−3.0	
P.E.	3.53	2.77	2.77	1.72	
Dev./P.E.	1.30	1.16	1.74	1.74	

Since four out of 20 anemics obtained were hairless, where we should expect none if anemia were closely linked with hairless, we may conclude that the bellyspot-anemia complex is independent of the piebald gene. The deficiency of the recombination double recessive class is only 1.74 times its probable error and may be attributed to chance or to a slightly higher mortality among anemic hairless mice.

In earlier data from this same cross, in which the F_2 classification was based on animals phenotypically flexed rather than upon the more reliable anemia counts, the distribution in table 10 was obtained.

TABLE 10

F_2 involving hairless and flexed-tail (repulsion).

	NORMAL		FLEXED		TOTAL
	Hr	*hr*	*Hr*	*hr*	
Observed	109	60	12	6	187
Expected 3:1			13.5	4.5	
Dev.			−1.5	1.5; P.E. 1.24; Dev./P.E. 1.21	

Although the expected number of flexed-tail mice did not appear, probably because of normal overlaps, 6 out of 18 which did appear were hairless. This is in close agreement with expectation, showing that flexed and hairless recombine freely. But since flexed is due to the same gene as bellyspot (or to one closely linked with it), and since hairless is closely linked with piebald, it follows that bellyspot and piebald must also be independent of each other (not linked).

Further evidence of the independence of bellyspot and piebald was obtained by crossing some of my bellyspot females with a selected strain of piebald produced by Dr. GREGORY PINCUS, which is phenotypically similar to my bellyspot race. Indeed, the two kinds of bellyspot look exactly alike, but that of PINCUS depends for its expression upon the presence of *ss* (the piebald gene). All the F_1 animals resulting from this cross were non-spotted, which in itself is strong evidence that the two spottings are genetically different. If the two are independent, we should expect in F_2 a ratio of 9 normal (non-spot):3 Pincus bellyspot:4 anemic (Clark bellyspot), and of these last, one would carry Pincus bellyspot in addition. The numbers recorded in table 11 were obtained.

TABLE 11

F_2 involving anemia (bellyspot) and a piebald bellyspot.

	NORMAL	SPOTTED NON-ANEMIC	ANEMIC	TOTAL
Observed	110	36	44	190
Expected 9:3:4	107	35.6	47.5	
Dev.	3.0	0.4	−3.5	
P.E.	4.61	3.63	4.03	
Dev./P.E.	0.65	0.01	0.87	

As can be seen, very good agreement with a 9:3:4 ratio was obtained, which is added proof that bellyspot (anemia) is not controlled by the piebald gene. Many of the F_2 spotted animals had dorsal spotting of some kind in addition to the ventral spot characterizing the grandparents. Some of them had a complete belt, isolated dorsal spots, a white nose, or a combination of all three. Some of these were anemics and some were non-anemics. Only eleven mice would be expected to be homozygous for both kinds of spotting, and doubtless some of the anemic individuals with dorsal spotting belonged to this class. The others were probably mice carrying a double dose of one spotting and a single dose of the other.

Tests for linkage between anemia (bellyspot) and dominant spotting, W

Bellyspot resembles dominant spotting (*Ww*) in that each has an anemia associated with it. But in the case of the former, the spotting is recessive, and the accompanying anemia is not lethal. It was thought barely possible that bellyspot, because of the anemia accompanying it, might be a recessive allele of dominant spotting. Since it is recessive to normal, we should hardly expect it to be allelic for physiological reasons.

If bellyspot were allelic, we should expect the following results from crosses with *Ww*. Let *w'* be used as the symbol for bellyspot, assumed, for the time being, to be an allele of *W*.

P_1 $Ww \times w'w'$
F_1 Ww' spotted, and ww' non-spotted

Mating the spotted F_2 *inter se* ($Ww' \times Ww'$), we should expect in F_1: 1 *WW* (which dies); 2 Ww' (spotted non-anemic); 1 $w'w'$ (bellyspot anemic). All the mice which were not anemic would have dominant spotting.

If bellyspot and dominant spotting are *not* allelic, we should expect very different results. Let bellyspot be represented by *ff*. We should then have:

P_1 $WwFF \times wwff$
F_1 *WwFf* (spotted) and *wwFf* (non-spotted)

Mating together the spotted F_2 (*WwFf*), we should expect the following types in F_2:

4 { 1 *WW FF* dies
  { 2 *WW Ff* "
  { 1 *WW ff* "

6 { 2 *Ww FF* non-anemic dominant spotting
  { 4 *Ww Ff* " " " "

3 { 1 *ww FF* non-anemic selfs
  { 2 *ww Ff* " " "

3 { 1 *ww ff* anemic (bellyspot)
  { 2 *Ww ff* " "

If the two spottings are independent, we should accordingly expect a ratio of 3 non-anemic selfs to 6 non-anemic spotted to 3 anemic, or a 1:2:1 ratio. In case dominant spotting and anemic bellyspot are alleles, we should not expect the non-anemic self class to be produced, since all the non-anemics would be spotted. Moreover, if the two were not allelic but linked, we should expect this first class to be smaller than indicated above.

Some bellyspot-flexed-anemic mice were crossed with a dominant spotted strain, and the F_1 spotted animals were mated *inter se*. The character of the F_2 generation is shown in table 12.

Fairly good agreement is observed with a 1:2:1 ratio which indicates that the two types of spotting are neither allelic nor linked as explained above. Since this is a repulsion cross, if bellyspot and dominant spotting were linked, more than two-thirds of the non-anemics would be spotted; in fact, the closer the linkage, the nearer the results would tend to be like those outlined in the hypothetical case of allelism on the previous page.

TABLE 12

F_2 involving dominant spotting and bellyspot-anemia (repulsion).

	NON-ANEMIC SELF	NON-ANEMIC SPOTTED	ANEMIC BELLYSPOT	TOTAL
Observed	10	25	16	51
Expected 1:2:1	12.7	25.5	12.7	
Dev.	−2.7	−0.5	3.3	
P.E.	2.09	4.72	2.09	
Dev./P.E.	1.29	0.11	1.58	

Further evidence of the free assortment of these two types of spotting is obtained when we mate the spotted F_1 (*WwFf*) with non-spotted F_1 (*wwFf*) animals from the same P_1 cross. In a repulsion cross of this kind, if these two characters were linked, we should expect to obtain very few, if any, anemic animals which have a face blaze characteristic of this particular strain of dominant spotting. In case linkage does not exist, we should expect the following types to be produced in F_2.

3 { 1 *ww* *FF* non-anemic self
  { 2 *ww* *Ff* " " "

3 { 1 *Ww* *FF* non-anemic-spotted
  { 2 *Ww* *Ff* " " "

1 *Ww* *ff* anemic dominant spotted

1 *ww* *ff* anemic bellyspot (or overlap for bellyspot)

In other words, a 3:3:1:1 ratio would be expected on the basis of no linkage. The observed result is shown in table 13.

TABLE 13

F_2 involving dominant spotting and bellyspot-anemia, obtained by crossing spotted F_1 with non-spotted F_1 (repulsion).

	NON-ANEMIC		ANEMIC		TOTAL
	SELF	SPOTTED	DOMINANT SPOTTED	BELLYSPOT	
Observed	23	28	6	9	66
Expected 3:3:1:1	24.75	24.75	8.25	8.25	
Dev.	−1.75	4.25	−2.25	0.75	
P.E.	2.96	2.96	1.81	1.81	
Dev./P.E.	0.59	1.10	1.24	0.41	

The close agreement in table 13 between observed and expected numbers indicates that bellyspot and dominant spotting are not linked. Belly-

spot, then, is a new recessive spotting factor in mice, since it shows free assortment with both dominant and piebald spottings.

If bellyspot is a new recessive spotting, it was thought desirable to test its linkage relations with other characters. Because of failure to obtain crossovers between bellyspot and anemia, the use of anemia as an indicator for presence of the bellyspot gene was continued. For the P_1 crosses, however, mice were used which phenotypically showed bellyspot. Since all bellyspot animals which appeared in the F_2 were always anemic, this method was proved to be accurate.

Linkage relations were tested between bellyspot-anemia and the following characters:

1. *Sex.* If anemia were a sex-linked character, we should expect, from the cross of anemic male with normal female, to obtain only normals in F_1, and in F_2 half the males should be anemic but all the females normal. In reality, of the 63 anemic mice produced in F_2, 31 were males and 32 females. This result rules out the possibility of sex linkage for the gene concerned in anemia. Further, if the character complex were sex-linked, all the F_1 males would be flexed-anemic when a flexed-tail ♀ was crossed with a normal ♂. But no flexed-tail males appeared in such crosses, which is additional evidence that the bellyspot-flexed-anemic complex is not sex-linked.

2. *Pink-eye.* Since PLATE's data indicated possible linkage of his kinky-tail mutation with pink-eye, this linkage test was of special interest. HUNT et al (1933) has shown, however, that flexed-tail is not linked with albinism. The data of table 14 confirm his results, since pink-eye and albinism are closely linked with each other.

TABLE 14

F_2 involving pink-eye and bellyspot (coupling).

	NORMAL		ANEMIC		TOTAL
	P	*p*	*P*	*p*	
Observed	132	40	42	14	228
Expected 9:3:3:1	128.2	42.8	42.8	14.2	
Dev.	3.8	−2.8	−0.8	−0.2	
P.E.	5.05	3.98	3.98	2.47	
Dev./P.E.	0.75	0.70	0.20	0.08	

3. *Roberts' pink-eye* (p_2). ROBERTS (1931) reported a new pink-eyed mutation captured in the wild. He states that the shade of pink in the eye was indistinguishable from that in the eye of the common pink-eyed variety, but that the coat color, while plainly agouti, was lighter than that of a pink-eyed black agouti.

This gene dilutes both the yellow and black (or brown) in agouti individuals. A mouse duplex for this gene and for extreme dilution ($c^d c^d p_2 p_2$) has very little color, pigment being eliminated from the ears.

ROBERTS (1933, unpublished observations) finds that his pink-eye is linked with agouti, the crossover percentage being about 21.

The F_2 results from a cross of Roberts' pink-eye and anemia are shown in table 15. Since the number of animals recovered in the two crossover classes is very close to the number expected, it may be concluded that Roberts' pink-eye and bellyspot anemia are not linked. Since Roberts' pink-eye and agouti are linked, it follows that anemia is not linked with agouti.

TABLE 15

F_2 involving Roberts' pink-eye and anemia (repulsion).

	NORMAL		ANEMIC		TOTAL
	P_2	p_2	P_2	p_2	
Observed	60	23	14	8	105
Expected 9:3:3:1	59.1	19.7	19.7	5.5	
Dev.	0.9	3.3	−5.7	1.4	
P.E.	3.43	2.70	2.07	1.67	
Dev./P.E.	0.26	1.22	2.11	0.84	

4. *Agouti.* For tests with agouti, the agouti allele, tan belly, was used. Since agouti is linked with Roberts' pink-eye, a test with one of these two characters is sufficient. At the time these data were obtained, however, the fact that agouti and Roberts' pink-eye were linked was unknown. In this cross, bellyspot-anemic animals were crossed with an inbred short-ear dilute brown-and-tan Bussey strain, and an F_2 population was obtained. The F_2 results are shown in table 16.

TABLE 16

F_2 involving anemia and tan belly (repulsion).

	NORMAL		ANEMIC		TOTAL
	a^t	a	a^t	a	
Observed	167	80	57	17	321
Expected 9:3:3:1	180.6	60.2	60.2	20.0	
Dev.	−13.6	19.8	−3.2	−3.0	
P.E.	5.99	4.72	4.72	2.93	
Dev./P.E.	2.27	4.19	0.68	1.02	

It will be noticed that the deviation in the second class is 4.19 times its probable error. Since this is a crossover class and the deviation is positive,

it does not indicate linkage. Moreover, the double recessive parental type (anemic non-agouti) did not appear in excess in F_2. We may conclude, then, that bellyspot anemia and agouti are not linked, thus corroborating the results obtained with Roberts' pink-eye.

5. *Dilution* (blue). The dilution used in this test came from the same inbred strain that was used in the previous cross. The F_2 results with anemia are shown in table 17. A very good fit to a 9:3:3:1 ratio was obtained, which indicates that bellyspot-anemia and blue dilution are located on different chromosomes. The largest deviation is in the double recessive class and is well within the limits of chance variation. It is quite possible that animals which have both dilution and anemia are more likely to die before time of classification.

TABLE 17

F_2 involving anemia and dilution (repulsion).

	NORMAL		ANEMIC		TOTAL
	D	*d*	*D*	*d*	
Observed	307	105	105	26	543
Expected 9:3:3:1	305	102	102	34	
Dev.	2.0	3.0	3.0	−8.0	
P.E.	7.80	6.13	6.13	3.80	
Dev./P.E.	0.26	0.49	0.49	2.11	

6. *Chocolate.* The data given in table 18, derived from the same cross indicate that anemia and chocolate are not linked. This time we have two classes which show a significant deviation from the expected numbers. If there were linkage, however, we should expect a large plus deviation in

TABLE 18

F_2 involving anemia and chocolate (repulsion).

	NORMAL		ANEMIC		TOTAL
	B	*b*	*B*	*b*	
Observed	244	42	78	21	385
Expected 9:3:3:1	216.6	72.2	72.2	24.1	
Dev.	27.4	−30.2	5.8	−3.1	
P.E.	6.57	5.17	5.17	3.20	
Dev./P.E.	4.17	5.84	1.12	0.97	

the parental classes and a large minus one in the crossover classes. This, however, is not the case. One of the large deviations is a positive one in the crossover class, normal black, and the other is a minus deviation in the parental class, normal brown. The other two classes, one parental and the

other crossover, show very good agreement with the expected numbers. We may conclude, then, that anemia and chocolate show independent segregation.

7. *Naked.* A male, heterozygous for naked, was crossed with an anemic-bellyspot female, and several of the F_1 naked males were crossed with bellyspot-anemic females. The results of this backcross are tabulated in table 19. Very close agreement with the expected 1:1:1:1 ratio was secured, indicating that the genes for naked and anemia are located on different chromosomes.

TABLE 19

Backcross involving anemia and naked (repulsion).

	NORMAL		ANEMIC		TOTAL
	Nk	*nk*	*Nk*	*nk*	
Observed	20	17	18	17	72
Expected 1:1:1:1	18	18	18	18	
Dev.	2	−1	0	−1	
P.E.	2.48	2.48	2.48	2.48	
Dev./P.E.	0.81	0.40	0.00	0.40	

8. *Rodless retina.* Two mice possessing the retinal eye defect, rodless, were secured from Dr. CLYDE E. KEELER and crossed with anemic animals. On the basis of free segregation, one would expect in the F_2 a ratio of 9 normal:3 non-anemic rodless:3 anemic non-rodless:1 anemic rodless. Or, considering the anemics alone (last two classes), we should expect one anemic out of four to be rodless. Since this is a repulsion cross, if these two characters were linked, less than one-fourth of the anemics should be rodless; and the stronger the linkage, the greater this deficiency would be. For this reason, I considered it sufficient to section the eyes of the anemics only and discard the non-anemics. The data secured by following this procedure are shown in table 20, and they show clearly that bellyspot-anemia and rodless are not linked.

TABLE 20

F_2 involving anemia and rodless retina (repulsion).

	NORMAL		ANEMIC		TOTAL
	R	*r*	*R*	*r*	
Observed	Discarded		27	6	33
Expected 3:1			24.75	8.25	
Dev.			2.25	−2.25; P.E. 1.68; Dev./P.E. 1.34	

9. *Waltzing.* An F_2 ratio obtained from a repulsion cross involving waltzing and anemia is shown in table 21. It will be noticed that the observed crossover classes fit very closely to the numbers expected on the basis of no linkage. Obviously, the genes for waltzing and bellyspot-anemia are borne on different chromosomes.

TABLE 21

F_2 involving anemia and waltzing (repulsion).

	NORMAL		ANEMIC		TOTAL
	V	*v*	*V*	*v*	
Observed	46	9	13	5	73
Expected 9:3:3:1	41.1	13.7	13.7	4.6	
Dev.	4.9	−4.7	−0.7	0.4	
P.E.	2.86	2.25	2.25	1.39	
Dev./P.E.	1.71	2.09	0.31	0.30	

10. *Brachyury* (short-tail). Brachyury mice obtained from Dr. HUNT were crossed with bellyspot anemic animals, and F_1 short-tailed individuals were crossed with anemics. The backcross data are shown in table 22. Although the first class shows rather a large deviation from the expected number, the other three observed classes agree very well with the calculated numbers. The data indicate clearly that brachyury and bellyspot-anemia are not linked.

TABLE 22

Backcross involving anemia and brachyury (repulsion).

	NORMAL		ANEMIC		TOTAL
	Br	*br*	*Br*	*br*	
Observed	65	48	53	49	215
Expected 1:1:1:1	54	54	54	54	
Dev.	12	−6	−1	−5	
P.E.	4.29	4.29	4.29	4.29	
Dev./P.E.	2.80	1.40	0.23	1.16	

11. *Leaden.* Several leaden bellyspot-anemic mice, obtained from Dr. JOSEPH MURRAY, were crossed with normal mice, and the F_1 animals were backcrossed with the leaden anemic parents. The results secured from this backcross are shown in table 23.

The two parental classes (non-anemic non-leaden and anemic leaden) deviate considerably from the calculated numbers, but these deviations are only 2.25 times their respective probable errors, and one is a plus, while the other is a minus, deviation. The crossover classes deviate still

TABLE 23

Backcross involving anemia and leaden (coupling).

	NORMAL		ANEMIC		TOTAL
	L	*l*	*L*	*l*	
Observed	45	43	31	29	148
Expected 1:1:1:1	37	37	37	37	
Dev.	8	6	−6	−8	
P.E.	3.55	3.55	3.55	3.55	
Dev./P.E.	2.55	1.70	1.70	2.25	

less from their expected numbers. Leaden and anemia, then, are independent characters, and are located on different chromosomes.

12. *Hydrocephalus.* Hydrocephalus is a new recessive mutation in the mouse (CLARK 1932, ZIMMERMANN 1933). Mice with this defect have a dome-shaped skull, caused by pressure of the cerebrospinal fluid within the ventricles of the fore-brain. Serial sections of the brain of newborn and older hydrocephalic animals show that this hydrocephalus is of the obstructive or internal type, produced by a congenital occlusion of the Aqueduct of Sylvius (results as yet unpublished). The cerebrospinal fluid which normally reaches the subarachnoid space is trapped in the fore-brain, and its accumulation there causes great distention of the lateral and third ventricles.

Hydrocephalus, in some cases, can be detected at birth but often can not be recognized with certainty until about the 15th day of postnatal life. Mice homozygous for this gene rarely live to sexual maturity.

Preliminary results seemed to indicate that this character was either linked with flexed-tail anemia or caused by the same gene. It is now certain that these two characters are not caused by the same gene. Linkage tests are in progress and will be reported at a later date.

13. *Dwarf.* Three mice heterozygous for dwarf (kindly supplied by Dr. E. C. MACDOWELL) were mated with bellyspot-anemic mice. The F_1 females were tested for dwarf by mating them with one or the other of two males from some of my other crosses which were known to be heterozygous for anemia and proved also to be heterozygous for dwarf. It was found that the pedigree of these two males traced back to the dwarf stock of Dr. SNELL. Dwarf animals appeared when they were mated with female sibs of the same ancestry. The F_2 results are shown in table 24.

The fit of the observed to the expected ratio, although it might be closer, shows clearly that anemia and dwarf are independent. Both parental classes (dwarf non-anemic and non-dwarf anemic) have fairly large deviations, but one of them is a plus deviation, while the other is minus. In

TABLE 24

F_2 involving anemia and dwarf (repulsion).

	NORMAL		ANEMIC		TOTAL
	Dw	*dw*	*Dw*	*dw*	
Observed	51	24	12	9	96
Expected 9:3:3:1	54	18	18	6	
Dev.	−3	6	−6	3	
P.E.	3.28	2.58	2.58	1.60	
Dev./P.E.	0.91	2.33	2.33	1.87	

each case the deviation is less than three times its probable error. The two crossover classes agree very well with the calculated numbers.

14. *Zavadskaia shaker* (DOBROVOLSKAIA-ZAVADSKAIA 1928b). This mutation arose among the descendants of X-rayed mice, and behaves as a Mendelian recessive. Animals with the defect exhibit constant nervous shakings of the head.

Before testing this character with bellyspot, it was considered desirable to demonstrate that it is not identical with either ordinary shaker or waltzing. When Zavadskaia shaker was crossed with waltzing, normal animals were produced in the F_1, proving that the two characters are different. In addition, Zavadskaia shaker mice, homozygous for chinchilla, were crossed with normal, fully colored mice. The F_1 were mated *inter se*, with the results shown in table 25. Since ordinary shaker is linked with

TABLE 25

F_2 involving Zavadskaia shaker and chinchilla (coupling)

	NORMAL		ZAV. SHAKER		TOTAL
	C	c^{ch}	*C*	c^{ch}	
Observed	21	8	12	2	43
Expected 9:3:3:1	24.2	8.1	8.1	2.7	
Dev.	−3.2	−0.1	3.9	−0.7	
P.E.	2.19	1.73	1.73	1.07	
Dev./P.E.	1.46	0.06	2.25	0.65	

chinchilla (albino series) with only 2 percent crossing over, this new shaker, if it were ordinary shaker, would also show linkage with chinchilla, but a good fit to a 9:3:3:1 ratio was obtained with no indication of linkage. After these results were obtained, DUNN informed me that he has crossed Zavadskaia shaker with both ordinary shaker and waltzing and finds that it differs genetically from both of them.

Preliminary results indicate that the Zavadskaia shaker character is not linked with bellyspot. The F_2 data are shown in table 26.

TABLE 26

F_2 involving anemia and Zavadskaia shaker (repulsion).

	NORMAL		ANEMIC		TOTAL
	*Sh*₂	*sh*₂	*Sh*₂	*sh*₂	
Observed	28	11	5	4	48
Expected 9:3:3:1	27	9	9	3	
Dev.	1	2	−4	1	
P.E.	2.32	1.82	1.82	1.13	
Dev./P.E.	0.43	1.98	2.20	0.88	

MUTANT CHARACTERS OF THE MOUSE

For the convenience of those interested in linkage studies in the mouse, I shall list the mutant characters of mice, indicating whether they are independent or linked, incompletely tested for linkage, extinct, or unfitted for linkage studies.

INDEPENDENT CHARACTERS OR LINKED GROUPS

Map distance

1. Agouti series 0.0
   Roberts' pink-eye 21.0 (ROBERTS)
2. Chocolate
3. Albino series 0.0
   Shaker 2.5 (GATES)
   Pink-eye 18.0 (HALDANE et al.)
4. Short-ear 0.0
   Dilution 0.034 (SNELL)
5. Leaden
6. Rodless 0.0
   Silver 12.0 (KEELER)
7. Piebald 0.0
   Hairless 8.0 (SNELL)
8. Dominant spotting
9. Bellyspot
   Recessive flexed-tail
   Anemia
10. Waltzing
11. Dwarf
12. Naked

Note: Leaden has not been tested for linkage with rodless or silver.

CHARACTERS NOT COMPLETELY TESTED FOR LINKAGE

13. Zavadskaia shaker
14. Dominant flexed-tail
15. Short-tail (brachyury)
16. Hare-lip and cleft-palate
17. Absence of *Corpus callosum*
18. Myencephalic blebs
19. Hydrocephalus
20. Hound-ear
21. Circling
22. Waved

CHARACTERS DESCRIBED BUT EXTINCT

23. Angora
24. Twisted nose
25. Inter-frontal
26. Parted frontals
27. Hyperglycæmia?

CHARACTERS WITH UNCERTAIN INHERITANCE PROBABLY NOT SUITED FOR LINKAGE STUDIES

28. Ectromelus
29. Polydactylism
30. Wildness

As can be seen from the above list, there are twelve characters, or groups of characters, which have been shown to be independent of each other. Leaden, although it has not been tested with rodless or silver, was provisionally included in this list. Thus, there is the possibility that only eleven chromosomes are accounted for, since leaden may be carried on the same chromosome as rodless and silver.

The second group includes ten characters which have been only partially tested for linkage or not tested at all. Undoubtedly some of these characters will prove to be linked with each other or with characters of the first group.

The third group contains five characters which have been described but are now extinct.

The fourth group includes three characters which have an uncertain inheritance, and are probably not suited for linkage studies, although opinion might differ on that point.

CONCLUSIONS

Bellyspot, a new spotting character in the mouse, is shown to be recessive, independent of piebald and dominant spotting, and very closely linked with the recessive flexed-tail-anemia complex reported by HUNT and PERMAR.

It is demonstrated that both bellyspot and flexed-tail give high percentages of normal overlaps, while anemia, the third member of the complex, behaves as a typical Mendelian recessive without normal overlaps.

Postnatal mortality is shown to be 10 percent higher among anemic mice than among normals during the first few weeks of life. No evidence was found to indicate that anemic-bellyspot mice have a higher prenatal death rate than normals.

One mutation to white belly was found.

Data are presented proving that bellyspot is not linked with piebald, dominant spotting, sex, pink-eye, Roberts' pink-eye, agouti, dilution, chocolate, naked, rodless, waltzing, brachyury, leaden, dwarf, or Zavadskaia shaker.

The bellyspot-flexed-anemic complex, therefore, is independent of the

eleven independent characters or linked groups in mice. With the completion of these tests, the number of mutually independent characters or linked groups is increased from eleven to twelve by the addition of belly-spot-anemia.

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A CYTOLOGICAL AND A GENETICAL STUDY OF PETUNIA V

THE INHERITANCE OF COLOR IN POLLEN[1]

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While many investigators have studied the problem of the inheritance of color in reference to the corolla, the fruit, and the seed in a considerable number of plants, only a few students of color in floral parts have made more than a casual reference to pollen color and its inheritance. The origin and distribution of color in the anther and in the pollen are to be considered in a later paper. At present we are concerned solely with the method of inheritance of pollen color.

In 1858, NAUDIN reported a series of experiments in hybridization which he had carried out during the summers of 1854, 1855 and 1856. His investigations were based on the two species, *Petunia violacea* and *Petunia nyctaginiflora*. He noted that the former has a blue throat associated with "blue violet" pollen, while the latter has "pale yellow" pollen and a throat tinted with yellow and marked with delicate "brownish" veins. He found the first generation hybrids of these two species very uniform in all respects, and intermediate between the two parents. The second generation hybrids, even when derived from seeds of the same capsule, were markedly variable. Notwithstanding the fact that he made no attempt to prevent promiscuous pollinations in the field, his results are of great interest, even though many observations recorded are not in line with recent findings. He did not report observations on any F_2 family which consisted of less than 10 mature plants; and because of lack of space he brought to maturity not more than 40 plants in any one of his second generation families. In one family of 25 plants, 8 bore yellow pollen and 17 gray or blue pollen. Evidently because colored flowers were much more abundant in his cultures than white flowers, he frequently made the statement that *Petunia violacea* produces a much stronger effect upon the hybrid progeny than does *P. nyctaginiflora*.

Some F_2 plants could not be distinguished from *P. violacea* in regard to morphological characters as well as to color of corolla and of pollen; while a lesser number of plants resembled in all respects, including yellow pollen, *P. nyctaginiflora*. Still other plants bore flowers of various shades of color, ranging from that of the colored parent to that of the white parent; but

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always the plants with blue pollen were in excess of those with yellow pollen. In some the limb of the corolla was almost white, the throat purple and the pollen gray blue. He mentions especially two plants of an F_2 family which bore lilac-tinted corollas, one having yellow pollen and the other gray-blue pollen. At this early date, then, the fact that pollen color and color of corolla limb are inherited independently in certain hybrids of Petunia was clearly demonstrated, though not asserted, by NAUDIN. In many of his later papers, 1863, 1865, et cetera, NAUDIN gave further data regarding the inheritance of color and other floral characteristics in Petunia, but he added practically nothing of value to his earlier observations in reference to the inheritance of pollen color.

So far as I have been able to determine, no other investigations regarding the genetics of pollen color in Petunia were reported until some sixty years later. In 1918 RASMUSON made a brief study of a cross between a petunia plant with flowers having white limbs, blue throats and blue pollen and a plant bearing flowers characterized by violet limbs, yellow throats and yellow pollen, the latter being used as the pollen parent. These plants were evidently chance variations of cultivated strains and, as is to be expected, the F_1 plants were variable. He decided that one parent at least must have been heterozygous. The F_1 plants fell into four groups according to the colors of limb, throat and pollen. From a consideration of but 42 plants which were distributed among these groups, RASMUSON concluded that blue anthers and blue pollen are dominant to yellow anthers and yellow pollen with a monohybrid segregation in the F_2. He further concluded, from an analysis of less than 50 plants, that with blue anthers and blue pollen the throat is always blue; that yellow pollen and yellow anthers are always associated with yellow throat; and that the gene for deep flower color and the gene for blue pollen are inherited independently.

We find in our cultures very clean-cut evidence that in certain hybrids the inheritance of limb color is entirely independent of that of throat, or of pollen color. Evidently the factors determining throat color and those controlling the color of pollen in these hybrids are not located on the same chromosome or chromosomes as are those responsible for limb color. Throat and pollen color, however, are always linked with a certain percentage of crossing over, so that pollen of a given color is usually, but by no means always, associated with a throat of a definite color. The genetics of throat color in relation to color of limb and to color of pollen is to be discussed in a paper to be published in the near future, and will not be considered further at this time.

The only other reference which I find regarding the inheritance of pollen color in Petunia is the statement recently made by DALE (1931). He affirms, as had RASMUSON earlier, that "pollen color in diploid petunias

shows monohybrid inheritance." The results of our observations, based both on species crosses and on crosses with certain cultivated strains which are homozygous for flower color, are not in accord with such a conclusion. DALE does not give the origin of his diploid petunias. Under usual conditions certain F_2 and F_3 diploid segregates would be expected to show a monohybrid inheritance. The species crosses, as well as those of the cultivated strains of Petunia which we are growing, indicate very clearly, as will be shown later, that several factors are concerned in the inheritance of pollen color. In accordance with our analysis, but 4 genotypes in every 256 F_2 plants of the species cross would breed true when selfed and give a monohybrid ratio for pollen color when selfed or when crossed with the homozygous recessive. These would comprise the yellow pollen of *P. axillaris*, 2 shades of green and 1 shade of blue.

METHODS AND MATERIAL

In order to secure unity in a series of records taken over a term of years and by different individuals, one of the first essentials is that there be worked out, as the result of preliminary experimentation, a definite method of procedure to be followed by all. It is a common observation that the flowers of petunia change color as they age; but we find that no marked differences in pollen color can ordinarily be detected between anthesis and the fading of the flower. Notwithstanding, it seemed desirable that the determination of all pollen color should be made between certain limits of time following the dehiscence of the anthers. As a rule the pollen of diploid petunias is shed on the same day as that on which the flower opens. When pollen is to be collected, all flowers are cut from the plants in the late afternoon of the day previous to that on which pollen is to be taken. Any flowers present on the next day must of necessity be freshly opened. If for any reason it is not desirable to remove all flowers, pieces of light twine are tied to the peduncles of such flower buds as would normally open the next morning. The same pollen varies appreciably in color depending on whether it is dry or moist. Collections of pollen are, therefore, never made in the early morning, on "foggy days," or directly after rain. Thus the pollen used in this study was both *fresh* and *dry*.

All the color determinations are based on Ridgway's Color Standards and Color Nomenclature. In matching the pollen to the standard colors, we found it necessary to have a uniform and carefully worked-out plan if results were to be, in any degree, dependable. Even with close adherence to the best of plans, we do not believe that errors, in assigning pollen to the intergrading hues of color which lie between the more clearly defined spectrum colors, can be entirely eliminated. As already stated, moist pollen has not the same shade or tint as has dry pollen; the hue may vary

appreciably with variations in the amount or depth of the pollen that is being considered; a given mass of dry, fluffy pollen will match to, or appear identical with, various tints or shades of color depending on the color of the background on which it rests. For instance, different tints or shades of yellow may be assigned to the same bit of yellow pollen if the pollen when being matched to the Ridgway colors rests on a dark yellow, a light yellow, a yellowish green, a gray or other colored background. After various trials we decided that the most satisfactory method is to place a small mass of pollen on or near the edge of a glass slide, and to hold the slide from an inch to an inch and a half above, with the pollen mass just at the margin of, the block of color with which the pollen is being compared. A piece of white cardboard with a rectangular opening the size of a single color block is placed over the Ridgway color plate so as to eliminate the influence on the eye of all colors except the particular one with which comparison is being made.

Since the intensity of light varies not only on different days but also at different hours of the same day, and since variations in light intensity change the tones of certain shades of color as registered by the eye, it has been our practice for some years to take all color records by means of a daylight lamp especially constructed for color-metric work, and in a darkened room, thus securing a certain degree, at least, of uniformity of light. We obtained from the publishers separate pages of their color plates which were most used in our work. With the particular design of lamp which we have, the lighted surface being an inclined plane enclosed on three sides, the separate plates are much easier to manipulate when one is taking records than is the book with its stiff covers. The co or plates are always placed in a definite position with regard to the center of the lighted surface in order that color determinations may be made under as nearly identical light conditions as possible.

In securing pollen, the corolla is cut away at a distance well beneath the anthers. By means of a needle or a spear the filaments are tapped causing the pollen to be shed on a glass slide placed just beneath the anthers. As the amount of pollen varies in the flowers of the different members of a hybrid family, it is sometimes necessary to use pollen from more than one flower of a given plant. It would be very simple to hold the recently dehisced anthers, with their pollen still adhering, directly over the blocks of color; but since the color of the anther, which may not be the same tint as that of the pollen, would influence one's judgment regarding the exact tone of the pollen color, such a procedure is not feasible.

The problem of "color matching" is further complicated by the fact that one's judgment varies at different times, so that one may match the pollen of a given plant to a certain hue or shade on one day and the pol-

len of the same plant to another, perhaps closely related, hue or shade on the following day or week. In addition to this, there is the well known fact that the human eye is quickly fatigued as the result of close application. For this reason, we do not plan to determine color tints or shades for longer than an hour at a sitting. It has been our intention to adhere very closely to the method, as outlined above, for the determination of pollen colors. But even so we are sure that the records which follow do not in all cases exactly conform to the various hues of color as they really exist in the plant. While it is usually very clear as to which of the large groups of spectrum colors a mass of pollen under consideration belongs, it is, in certain instances, equally difficult to decide as to just which tint of the Ridgway colors certain samples of pollen correspond. The greatest difficulty which we have encountered is in distinguishing between greenish yellow and yellowish green tints. To illustrate, it is, as a rule, well-nigh impossible to decide whether a certain tone of pollen should be listed as Pale Green Yellow or as Pale Viridine Yellow. Yet if assigned to the former tint it would fall in the yellow group, if to the latter tint, in the green group. In our final summary, we have placed one-half of the plants assigned to these two colors in the yellow group and one-half in the green group. This seems to us the only feasible solution, since the decisions here are so largely a question of judgment, and since during the last ten years records of pollen color have been taken not only by the writer but also by three assistants. Fortunately these tints do not often occur in the pollen of the plants under observation and, therefore, would not materially affect the ratios when large numbers are being considered. While recognizing such possibilities of error, we still feel confident that the ratios as obtained for the large groups of spectrum colors would not be substantially changed were it possible to eliminate every vestige of error. Since different people do not see colors exactly alike, we have been guided, in assigning the individual shades or tints to the groups of spectrum colors, and in listing them within these groups, by "the relative proportions of two or more colors in any mixture" as given by Ridgway.

The record of observations and the discussions which follow are based on the color behavior of the pollen in two distinct lines of hybrids. One series of F_1, F_2, and F_3 plants, together with the backcrosses to the parents and the intercrosses between members of the same F_2 family, was derived from crossing *Petunia axillaris* (Lam.) BSP. (*P. nyctaginiflora* Juss.) with *Petunia violacea* Lindl. The second series of hybrids had its origin in crosses between *P. axillaris* and a large-flowered garden variety with rich deep-purple corolla and Turtle Green pollen. This cultivated strain is known in our cultures as population eight (P8). We have grown this strain of petunia since 1919 and it has continued to breed true to color of floral parts for ten

generations. Unfortunately it was attacked by mosaic and during the winter of 1933 was completely lost to our cultures. A certain lessening of vigor as the result of long inbreeding may have increased its suceptibility, and its lack of resistance, to disease. We are inbreeding certain segregates from its crosses with *P. axillaris* and also segregates from crosses between it and another strain of deep purple-flowered petunias. It is too early to report the results of these efforts to segregate out a plant of the same genotype, in regard to floral characteristics, as the original strain. Records have been taken of pollen color for more than 22,000 plants of these two series.

There is every reason to believe that the two species considered in this study are the same as those described by NAUDIN in 1858 and used in his experiments. We recently redescribed and traced the history of these two species (FERGUSON and OTTLEY 1932). Not until our paper had been sent to press did the name and description of *Petunia Parodii* (STEERE 1931) appear. It is evident when one compares STEERE's description and illustrations (Plates XXXII and XXXIII) of *P. Parodii* with *P. axillaris* (Lam.) BSP., as described and illustrated by us in 1932, that the entity is one and the same for the two names. The concept accepted by STEERE for *P. axillaris*, as judged from his illustrations and text, agrees markedly with the cultivated strain, which we raised, in 1932, to the dignity of a horticultural species, assigning to it the name *Petunia alba*. We have not succeeded in finding any statement by STEERE regarding the origin of the plants which he accepts as *P. axillaris*. This information is undoubtedly given in an earlier paper but, if so, it has been overlooked by the writer.

Petunia violacea Lindl. is known in our cultures as population nine (P9) and *Petunia axillaris* (Lam.) BSP. as population fourteen (P14). The former has Cadet Gray pollen, the latter Pinard Yellow. The color records for the series involving the two species[2] have all been made in a darkened room by means of the daylight lamp. The earlier records for the series in which P8 was one of the original parents were taken on sunny days in the greenhouse and in the field, always with the back of the recorder so placed as to secure as nearly as possible north light, and never with the flower in direct sunlight. During the last four years color records for both series have been taken in a darkened room, by means of the special lamp to which reference has already been made, and with the pollen mass on a glass slide as described earlier.

As stated in 1932, we did not succeed in securing plants of *Petunia violacea* Lindl. until late in the summer of 1930. This species is rather reluctantly self-fertile, having been grown at Kew Gardens, the source of our plants, for 16 years without setting seed. Some experimentation was neces-

[2] I take pleasure in giving most cordial recognition of the very efficient work of BARBARA HUNT, my special assistant in carrying out the investigations on this species cross.

sary before seeds were secured as the result of selfings, and a year elapsed before the species was established in our cultures in sufficient numbers to enable us to use it freely in the work of hybridization. The largest number of seeds yet obtained, in a given capsule, is 82, whereas capsules resulting from selfing *P. axillaris* may contain over 1000 seeds. Also the percentage of germination is low, rarely exceeding 50 percent, often considerably less. This refers to the F_1 and F_2 families of the crosses of P9 with P14 as well as to capsules resulting from selfing P9. For these reasons, the records of pollen colors for the crosses between P9 and P14 and later generations are much fewer in number than are those of the series of hybrids resulting from the various crosses involving P14 and P8. However, since *Petunia violacea* (P9) and *Petunia axillaris* (P14) have been universally accepted as the species which, through free intercrossing under cultivation, have given rise to the "garden petunias" of all countries, it seems desirable that the species crosses and their progeny should be considered before taking up the crosses involving the cultivated strain, even though the report does not cover so large a number of plants.

PETUNIA AXILLARIS (Lam.) BSP.(P14) × PETUNIA VIOLACEA LINDL. (P9)

As indicated in an earlier paper (BROOKS, WALSH and FERGUSON 1930), the pollen of P14, while very active on its own stigmas and to a lesser degree on those of its own sibs, is almost 100 percent inactive on the stigmas of all other species that we are growing and of all cultivated strains in our cultures not immediately related to it. For this reason, P9 is the pollen parent for all the F_1 plants which we have grown in this series. Backcrosses to both parents have been made with comparatively larger families resulting from the crosses between P14 and the F_1 than those resulting from crosses between the F_1 and P9.

In an attempt to explain and to correlate our observations on the various crosses involved we have assumed that 4 pairs of allelic factors are operative in the inheritance of color in the pollen of Petunia.

B is a factor for blue and is dominant; *b* gives very delicate tints of blue.

Y is a factor for yellow and acts as an incomplete dominant. The recessive, *y*, gives very light tints of yellow, and *Yy* is intermediate in its color reactions between *YY* and *yy*.

B and *Y* together produce green. Yellow color is possible only in the absence of *B* and likewise blue color can occur only in the absence of *Y*; but the dominant of the one prevails over the recessive of the other, as *B* over *y* and *Y* over *b*. The combination *by* gives very delicate shades of green.

D is a factor for gray and is an incomplete dominant, *Dd* being intermediate in its reactions between *DD* and *dd*. The recessive, *d*, gives white and acts only as a dilution factor. *D* cannot express itself as gray under any circumstances, and acts only to tone down or dull the other colors.

G is a factor for green and produces, phenotypically, a different tone of green than that given by the association of genes *B* and *Y*. It is a partial dominant; but *Gg* is only slightly weaker in its color reactions than *GG*. *G* is hypostatic to both *B* and *Y* and, therefore, the difference between *GG* and *Gg*, as agents of pigmentation, becomes apparent, only when both *B* and *Y* are absent. In the absence of *D*, *G* gives a greenish cast to the yellows and deepens the greens and the blues, but it cannot express itself as green except in the entire absence of both *B* and *Y*. The recessive, *g*, produces very pale green.

It follows that the various spectrum colors and the intergrading hues depend for their expression not only on the amount or dosage of each color factor but also on the reactions of these factors to one another. The factorial analysis, as above stated, is at best but a clumsy attempt to express the many and the more refined reactions that undoubtedly take place in petunia plants in the development and expression of color in floral parts.

Based on the assumptions as outlined above, the Cadet Gray pollen of *P. violacea* is represented by *BBDDGGyy*, the Pinard Yellow of *P. axillaris* by *bbddggYY*, and the Greenish Glaucous-Blue pollen of the F_1 by *BbDdGgYy*. Records have been taken of the pollen color of 5 families of first generation hybrids comprising 237 plants, and we have never observed a single instance of color other than that of Greenish Glaucous-Blue which, as will be seen by reference to table 1, is a shade of green. This is not then a case of clean-cut dominance as affirmed by the writers referred to earlier. The F_1 pollen is not the color of that of either parent; rather it is a new color which may possibly be interpreted as intermediate between that of the two parents, although it actually contains neither orange nor yellow. Apparently the orange and yellow pigments of the Pinard Yellow (P14) have so reacted with the blue, green, and gray pigments of the Cadet Gray (P9) as to produce the grayish blue-green pollen of the F_1. It is found that a considerable range of color occurs in the pollen of the second generation hybrids. The assumptions which we have stated provide for at least 29 shades or tones of color in the F_2 plants. These are distributed within three groups of spectrum colors — blue, green, yellow.

It is not to be expected that all these intergrading color tones would be detected, and in their relative frequency, without observing a very large number of plants; especially would such an expectation not be entertained when one considers the low percentage of seed germination in this series in connection with the fact that in practically 15 percent of these F_2 plants there was a complete absence of pollen. In some entire families the color records in this series are based on the second flower that appeared on a plant under normal conditions and, because of space limitations, the

plant was destroyed immediately following the taking of its records. These plants, recorded as producing no pollen, would have undoubtedly, in many cases, yielded pollen at some later time had we been able to furnish space for their continuance. With rare exceptions, their anthers were normal in size and structure but devoid of pollen. In addition, one must bear in mind that the conditions governing the action and interaction of the color factors, as assumed, are such that 5 tints of color would normally appear but once each in 256 plants, while one shade may appear 48 times. The other colors vary in the possibility of their appearance from 2 to 24 times in every 256 plants; and no F_2 family yet obtained in this cross has yielded one-fourth that number of mature plants. It was, therefore, not without surprise mingled with satisfaction that we found, on summarizing our data for these F_2 plants, a very close approximation to the theoretical results imposed by our assumptions. A classified list of the shades and tints of color which we have recorded for these F_2 hybrids is given in table 1.

One cannot ignore this series of colors on the assumption that the great variation in tones of color is due to external or ecological factors. We have grown F_2 plants in acid, in alkaline and in practically neutral soils; under varying degrees of moisture, light, and temperature; in the greenhouse and in the open. According to our records, only the colors listed have occurred under the various cultural conditions and, to date, no others. We found the color of corolla limb often very markedly influenced by the changing conditions; but if there was any change in pollen color we were unable to detect it. In flowers developed in absolute darkness, the pollen still has color but it is very much lighter in tone or shade. The evidence seems clear that the colors herein recorded are of genetic origin and point conclusively to a series of complex reactions within a group of color factors. To attempt to determine by selfings and crosses the exact genotype or genotypes of each shade or tint of color would be a time-consuming task that appears to us of doubtful value. We have, therefore, sought only such an explanation of the facts observed as will satisfactorily account for the ratio between the number of plants falling in each of the three main groups of spectrum colors and at the same time provide for an adequate variation in the hues of color which fall within the spectrum colors.

Records have been taken for 30 F_2 families comprising a total of but 684 plants. Of these, 102 plants were characterized by an entire absence of pollen. On the basis of the factorial analysis which we have suggested, the number of plants in each of the three color groups given in table 1 should be in the proportion of 48 blue to 160 green to 48 yellow. The ratio of the number of plants, and the number of tints, in the larger groups of spectrum colors as observed, compared with that computed, is given in table 2.

TABLE 1

*A complete list of colors occurring, in our cultures, in the pollen of the F_2 generation of P. axillaris × P. violacea, and the composition of each tint as determined by Ridgway.**

YELLOW				
	Percent			
Baryta Yellow	25 Orange+75 Yellow	*O-YY*	21	f
Pinard Yellow	25 Orange+75 Yellow	*O-YY*	21	d
Massicot Yellow	25 Orange+75 Yellow	*O-YY*	21′	f
Naphthalene Yellow	100 Yellow		23′	f
Barium Yellow	100 Yellow		23′	d
Sulphur Yellow	75 Yellow+25 Green	*YG-Y*	25	f
Seafoam Yellow	75 Yellow+25 Green	*YG-Y*	25″	f
Pale Green-Yellow	55 Yellow+45 Green	*G-Y*	27	f
GREEN				
	Percent			
Pale Viridine Yellow	39 Yellow+61 Green	*GG-Y*	29	f
Glass Green	39 Yellow+61 Green	*GG-Y*	29″	d
Gnaphalium Green	39 Yellow+61 Green	*GG-Y*	29′′′′	d
Pale Turtle Green	24 Yellow+76 Green	*Y-G*	31″	d
Light Turtle Green	24 Yellow+76 Green	*Y-G*	31″	d
Pale Fluorite Green	11 Yellow+89 Green	*GY-G*	33″	f
Light Fluorite Green	11 Yellow+89 Green	*GY-G*	33″	d
Clear Fluorite Green	11 Yellow+89 Green	*GY-G*	33″	b
Pale Olivine	100 Green		35″	f
Dull Opaline Green	93 Green+7 Blue	*GB-G*	37′	f
Bluish Glaucous	93 Green+7 Blue	*GB-G*	37′′′	f
Deep Bluish Glaucous	93 Green+7 Blue	*GB-G*	37′′′	d
Pale Dull Glaucous-Blue	75 Green+25 Blue	*BB-G*	41′′′	f
Greenish Glaucous-Blue	75 Green+25 Blue	*BB-G*	41′′′	b
BLUE				
	Percent			
Pale Russian Blue	45 Green+55 Blue	*BG-B*	45′′′	f
Russian Blue	45 Green+55 Blue	*BG-B*	45′′′	d
Cadet Gray	45 Green+55 Blue	*BG-B*	45′′′	b
Pale King's Blue	25 Green+75 Blue	*G-BB*	47′	f

* The symbols *O-YY*, *YG-Y*, etc. indicate the color relations in the color mixtures of each tint. The figures 21–47 indicate the steps in the spectrum scale.

The letters b, d, and f indicate the presence, respectively, of 9.5, 22.5 and 45 percent of white. The absence of a letter indicates full color.

The exponents ′, ″, ‴, and ′′′′ indicate the presence, respectively, of 32, 48, 77, 90 percent of neutral gray.

White weakens the spectrum color, gray dulls it.

It will be seen by reference to table 2 that there is in this instance a very close agreement between the actual observations and the calculations based on the assumption, as given earlier, of four allelic pairs of factors.

TABLE 2

Ratio of the number of plants in the 3 color groups, and the number of tints within each of these groups, in the pollen of the F_2 plants of P. axillaris×P. violacea.

Colors	RATIO OF NUMBER OF PLANTS				NUMBER OF TINTS		
	Blue	Green	Yellow		Blue	Green	Yellow
	101	379	102	Actual	4	14	8
	109	364	109	Calculated	4	17	8

Reciprocal crosses have been made between *P. violacea* and the F_1 plants of this series. The families in which the F_1 was used as the pollen parent have not yet flowered so that we can report at this time only on the cross of F_1(P14×P9)×P9. Evidently it is immaterial so far as fruit and seed formation is concerned, whether *P. violacea* or the F_1 acts as the pollen parent. Ten families, comprising 209 plants, have been considered. Of these plants, 106 yielded no pollen. We have, therefore, but 103 records of pollen color for the 10 families observed. Theoretically the plants in this backcross should bear green and blue pollen in the ratio of 1:1. The actual and the computed results are given in table 3.

TABLE 3

Ratio of the number of plants in the 2 groups of color, and the number of tints within each of these groups, in the backcross of F_1(P14×P9) to P. violacea and to P. axillaris.

Color	RATIO OF NUMBER OF PLANTS			NUMBER OF TINTS		
	Blue	Green	Yellow	Blue	Green	Yellow
F_1×P9	52	51		2	2	
	51.5	51.5	Calculated	2	2	
P14×F_1		189 : 453	Actual		6 — 7	
		321 : 321	Calculated		6 — 6	

Pollen color has been recorded for but one backcross to the F_1 in which P14 was used as the pollen parent. In this family but 7 plants matured. Because of the smallness of the family it was not included in this report. In general, we have not reported records of families yielding less than 10 plants, although the indications are that the ratios would not have been materially changed had the size of family been entirely ignored. The 7 families in which the F_1 was the pollen parent and *P. axillaris* the female parent gave rise to 642 mature plants and not one was characterized by abortive pollen. This backcross should give rise to plants bearing yellow and green pollen in the ratio of 1:1.

By reference to table 3, it is at once apparent that, in the backcrosses of the F_1 to *P. violacea*, both the actual ratios of plants and the number of

tints in the two color groups are in complete accord with the theoretical expectation. The unexpected excess of plants bearing yellow pollen in the backcrosses involving *P. axillaris* is interesting in connection with the unique type of sterility, referred to earlier, that is exhibited by *P. axillaris*. The anthers of P14 do not dehisce until after the corolla expands; but the pollen is apparently mature by the time that the flower bud has reached half the length that it attains before opening. One must, therefore, emasculate when the buds are comparatively small since later a slight pressure causes the anthers to burst, and some degree of selfing is almost sure to result. These backcrosses were made during the past three years, and, because of earlier experiences, the greatest of care was exercised in emasculating. We are, humanly speaking, certain that the great preponderance of yellow pollen in the progeny of this backcross has resulted neither from carelessness nor accident. The nature of the sterility displayed by *P. axillaris* together with the excess of yellow pollen recorded for this cross, suggests very strongly the presence in this species of selective fertilization. There may be some special physical or chemical attraction between the genes which unite at the time of fertilization in P14 to produce yellow pollen. So strong is this affinity that the pollen of *P. axillaris* does not function in fertilization in the absence of the possibility of the gene for yellow meeting another gene for yellow. In this case the eggs of P14 have equal chances to unite with sperms bearing the factor *B* or *Y*. It may be that the pollen tubes carrying the paternal cells which bear the factor *Y* are accelerated in their growth and penetrate the ovules in advance of the tubes with sperms carrying the factor *B*. This being so, a large majority of the seeds formed would carry the possibility of yellow pollen. On the other hand, it may be that there is a lack of affinity for, or that some form of repulsion exists between the gene for yellow and that for blue, or between the factor for yellow in P14 and certain combinations of factors occurring in the gametes of the F_1.

In this backcross, 12 different tints of color are possible, 6 in the green and 6 in the yellow group. Although the proportion of plants bearing yellow pollen to those bearing green in this backcross was found to be far removed from expectation, the number of color tints listed in the cross, as shown in table 3, more nearly accords with the number obtained theoretically. Of the 7 tints of yellow and 6 tints of green actually recorded, as also in the 4 colors registered as occurring in the backcross to *P. violacea*, no color was observed which is not listed in table 1 as appearing in the F_2 generation. The presence of so large a number of colors in these backcrosses gives further evidence that more than a single pair of allelic factors is involved in the production of pollen color in the two species of *Petunia* under consideration.

The activities of the color factors are such that but 5 of the 29 colors which may occur in the second generation of this cross could be depended upon to breed true genetically; of the others, 9 tints would or would not breed true depending on the exact genotype selfed; and the remaining 13 colors could not be expected to breed true under any condition. A considerable number of F^3 families are being grown, but only 13 of them can be reported on at this time. Of these, 1 family, from an F_2 parent with Cadet Gray pollen, has bred true to the parental color; of 3 families from Naphthalene Yellow pollen, 2 have bred true and 1 produced pollen of various shades of yellow; 4 families, each from a different shade of yellow pollen, have produced only yellow pollen, but in varying shades; 3 families from different shades of green gave, each, pollen in shades of green and yellow; one family from Seafoam Yellow pollen has, to date, given only yellow and blue pollen; and a family from Pale Dull Glaucous-Blue pollen has given rise to blue and green pollen only. These results will be referred to again later. The study of the pollen of the F_3 generation of this cross must progress much farther before any adequate basis is secured for assigning the various tints of color in the F_2 plants to one or more specific genotypes.

PETUNIA AXILLARIS (Lam.) BSP.(P14)×P8

The pollen color of P8, the deep-purple cultivated strain, is Turtle Green. When this strain is crossed with P14, the pollen of the F_1 generation is invariably Light Turtle Green. Here again we have incomplete dominance, though a superficial observation might lead one to conclude that the pollen color is the same in the F_1 as in P8 since both are green. The F_1 pollen differs from that of P8, as based on Ridgway, only by the presence of a larger percentage of white; but this lighter green is very constant and characteristic. It may be considered as an intermediate color, but it has no additional yellow over that of P8, and none of the orange of P14 in its composition. The new color evidently results from a definite set of chemical reactions between the genes controlling pigment color in the pollen of *P. axillaris* and those genes responsible for pigmentation in the pollen of P8. In this cross, also, P14 of necessity served only as the female parent. But it was active as the pollen parent in the backcross to the F_1 generation.

The question at once arises as to the origin of the green pollen of P8. It is generally believed today that *P. axillaris* and *P. violacea* were lost to cultivation, having been superseded by their more attractive hybrids, some years before the close of the last century, and that they again attracted the attention of the gardener only in comparatively recent times. Strains of petunia breeding true for green pollen today may have arisen many years ago as the result of mutations in either one or the other of the original species, or in their cultivated progeny, at some time since these

species were first hybridized. On the other hand, they may be survivors of those early F_2 hybrids which normally bore green pollen.

If, following the assumptions given earlier, one determines the constitution of the 81 genotypes occurring in the F_2 generation of a cross between *P. axillaris* and *P. violacea*, it will be seen that certain individuals are possible in which the dominant factor for blue pollen does not appear. It is not only possible but very probable that our cultivated strain, breeding true for green pollen, had its origin in such a genotype. Among the many thousands of plants resulting from crosses between *P. axillaris* and P8 that we have grown, not a single F_1, F_2, F_3, or F_4 plant has ever been found to bear blue pollen. We have, therefore, suggested that our large-flowered purple petunia (P8) is a direct descendant of an early hybrid of the genotype *bbDDGGyy*. But permanently removed from all influences on pigmentation exerted by the factor *B*, with which the factors *D* and *G* were originally associated in *P. violacea*, and under the varying conditions imposed by the gardener during years of cultivation, definite changes in the reaction of these color factors have taken place. Based on evidence from our cultures, it becomes apparent that certain mutations have occurred which affect both the actions and the interactions of the genes involved.

D, *G* and *Y*, as in the species cross, continue to act as incomplete dominants. However, *G* has assumed a more active rôle in pigmentation and is no longer hypostatic to *Y*; but homozygous *Y* is now revealed as a stronger pigment factor than homozygous *G*, so that *GGYY* produces yellow pollen with a green tone. On the other hand, due to the slight degree of incomplete dominance of *G* as compared with that of *Y*, *GG* or *Gg* with *Yy* produces yellowish green tints. The homozygous recessives, *ggyy* like the homozygous dominants, give a greenish yellow, which, in the case of the recessives, is a very light tint.

The change in *D* has been very slight and only such as to emphasize, somewhat, its activity in pigmentation. When homozygous, and in the absence of all other dominants, *D*, in its slightly modified rôle, gives rise to gray pollen.

The recessives remain unchanged in their influence on pollen color with the exception of *b* which has been practically reduced to white and serves only as a weak dilution factor.

The number of chromosomes in P8 is the same as in the species and there are no morphological differences, which can be detected by use of the microscope, between the individual chromosomes of *P. axillaris*, or those of *P. violacea*, and the chromosomes of the cultivated strain. The modifications which have taken place in the genes *b*, *D*, and *G*, as indicated above, are apparently not due to chromosome changes. They are rather to be considered as the results of gene mutations, the mutations having to do chiefly with the intensity of the reactions of the genes affected.

When P14 with its Pinard Yellow pollen (*bbddggYY*) is crossed with P8 with its Turtle Green pollen (*bbDDGGyy*), the F_1 pollen is Light Turtle Green in color and is represented by the genotype *bbDdGgYy*. The pollen of the F_2 plants shows as great a range of variability in color as that exhibited by the pollen of the second generation of the species cross. In this case, as noted earlier, there is an entire absence of blue and the various shades and tints of color fall into the three groups — green, yellow, gray. The various tints recorded as occurring within each of the main color groups in the F_2 plants of this cross and the composition of each tint, as based on Ridgway, is given in table 4.

TABLE 4

A classified list of the colors observed in the pollen of the F_2 generation of P14×P8, and the composition of each shade and tint as determined by Ridgway.

YELLOW				
	Percent			
Baryta Yellow	25 Orange+75 Yellow	*O-YY*	21	f
Pinard Yellow	25 Orange+75 Yellow	*O-YY*	21	d
Massicot Yellow	25 Orange+75 Yellow	*O-YY*	21′	f
Martius Yellow	100 Yellow		23	f
Naphthalene Yellow	100 Yellow		23′	f
Barium Yellow	100 Yellow		23′	d
Sulphur Yellow	75 Yellow+25 Green	*YG-Y*	25	f
Pale Greenish Yellow	75 Yellow+25 Green	*YG-Y*	25	d
Light Greenish Yellow	75 Yellow+25 Green	*YG-Y*	25	b
Pale Chalcedony Yellow	75 Yellow+25 Green	*YG-Y*	25′	f
Seafoam Yellow	75 Yellow+25 Green	*YG-Y*	25″	f
Pale Green-Yellow	55 Yellow+45 Green	*G-Y*	27	f
GREEN				
	Percent			
Pale Viridine Yellow	39 Yellow+61 Green	*GG-Y*	29	f
Light Yellow Green	24 Yellow+76 Green	*Y-G*	31	d
Pale Turtle Green	24 Yellow+76 Green	*Y-G*	31″	f
Light Turtle Green	24 Yellow+76 Green	*Y-G*	31″	d
Turtle Green	24 Yellow+76 Green	*Y-G*	31″	b
Light Fluorite Green	11 Yellow+89 Green	*GY-G*	33″	d
Clear Fluorite Green	11 Yellow+89 Green	*GY-G*	33″	b
Pale Olivine	100 Green		35″	f
Dull Opaline Green	93 Green+ 7 Blue	GB-G	37′	f
Dark Bluish Glaucous	93 Green+ 7 Blue	*GB-G*	37‴	b
Greenish Glaucous-Blue	75 Green+25 Blue	*BB-G*	41‴	b
Bluish Gray-Green	75 Green+25 Blue	*BB-G*	41‴	
GRAY				
	Percent			
Gull Gray	58.5 Black+41.5 White	Carbon Gray		d
Deep Gull Gray	67.5 Black+32.5 White	Carbon Gray		b

Records have been taken for 60 F_2 families of this cross totaling 7153 plants. Of these, 673 bore no pollen while 6480 produced pollen in abundance. On the basis of our assumptions the number of plants in each of the three groups of colors should be in the ratio of 108 yellow to 144 green to 4 gray. These F_2 plants should give rise to 27 shades and tints of color. The ratio of the number of plants, and the number of tints, occurring in each of the three groups of color, as compared with that expected is given in table 5.

TABLE 5

Ratio of the number of plants in the 3 groups of color, and the number of tints within each of these groups in the pollen of F_2 (P14×P8) plants.

RATIO OF NUMBER OF PLANTS					NUMBER OF TINTS		
Color	Gray	Green	Yellow		Gray	Green	Yellow
	99	3472	2909	Actual	2	— 12	— 12
	101	3645	2734	Calculated	1	— 12	— 14

The results, as given in table 5, show a rather broad discrepancy between the actual and the expected ratios. But this is not surprising when one bears in mind that they include the first plants for which records of pollen color were taken. A considerable proportion of the records for this cross was, therefore, made before the more refined methods, outlined earlier, for matching pollen to the Ridgway colors, had been adopted. Undoubtedly there was a failure in the early observations to distinguish clearly between certain greens and yellows.

In 2 of the backcrosses made between P8 and F_1(P14×P8) the F_1 served as the pollen parent and in 6 other cases the pollen was furnished by P8. The 8 families resulting from these pollinations comprised 973 plants of which but 777 were pollen-bearing. These plants should produce green pollen only and in 8 different shades. It will be seen by consulting table 6 that the theoretical expectations correspond perfectly in this cross with the results obtained. Our first backcross in this series was made in 1926. In this instance, P8 was used as the pollen parent. An exceptionally large family resulted. Of the 442 plants that produced pollen, 210 bore various shades of yellow pollen and 232 produced green pollen in varying shades. These results are more nearly what would be expected in an F_2 family and are manifestly due to careless manipulation in pollination. For this reason, together with the fact that the other 8 families gave only green pollen, the data for this largest family were not included in the summary of this backcross as given above and in table 6.

In the backcrosses to *P. axillaris*, the F_1(P14×P8) served as the pollen parent for 7 families and P14 for 1 family. These 8 families produced 3008 pollen-bearing plants which were divided into 2 groups as regards pollen

color. These plants should bear yellow and green pollen in the ratio of 3 to 1 and there should be 2 shades of green and 6 shades of yellow. The actual and the expected results in this cross are given in table 6.

TABLE 6

Ratio of the number of plants in the color groups, and the number of tints within each of these groups, in the backcross of P14 and P8 to F_1 (P14×P8).

	RATIO OF NUMBER OF PLANTS				NUMBER OF TINTS		
Color	Yellow		Green		Yellow		Green
P14×F_1	2271	:	737	Actual	6	—	2
	2256	:	752	Calculated	6	—	2
P8×F_1	0	:	777	Actual	0	—	8
	0	:	777	Calculated	0	—	8

The very close parallel between the results actually obtained in our cultures for the backcrosses of this series of hybrids, as shown in table 6, and the theoretical expectations further substantiate our assumption that at least four pairs of allelic factors are involved in the inheritance of pollen color in the two species *P. axillaris* and *P. violacea;* and that the cultivated strain with green pollen may represent an early segregate from a cross between these two species.

Following out the factorial analysis herein presented, in an attempt to explain the method of inheritance of pollen color in Petunia, it becomes clear that in some cases a given color may be represented by several genotypes, and thus, as stated earlier, may or may not breed true. To test this point, each plant of an entire F_2 family was selfed, resulting in over 8000 plants. The results show very conclusively that a given color of pollen may be represented by two or more genotypes. To illustrate, of 27 families secured from plants bearing Turtle Green pollen, 5 bred true to Turtle Green, 2 gave only green but in varying shades, 3 produced shades of yellow and green, and 17 produced, each, plants with a full range of yellow, green, and gray pollen. These 27 families were all secured from different plants of the same F_2 family. Only 2 plants of this F_2 family bore Greenish Glaucous-Blue pollen. These were selfed and both F_3 families came true to the parental color for pollen. It will be recalled that the pollen of the F_1 plants resulting from crossing P14 and P9 is Greenish Glaucous-Blue. All the factors for pollen color must, in this particular case, be heterozygous and the plants could not breed true for pollen color. These results indicate that one would need to repeat tests many times, in seeking to determine the constitution of the various phenotypes for pollen color, before one could be sure that the particular phenotype under consideration was repre-

sented by a single genotype, homozygous for all its factors, and by no other genotypes. Additional evidence of this same fact was given earlier in regard to the F_3 plants of P14×P9.

The record of observations as outlined and discussed in this paper would seem to justify the conclusion that at least 4 allelic pairs of factors are operative in the production and inheritance of pollen color in the F_1 plants, resulting from crossing *Petunia axillaris* and *Petunia violacea*, and in certain of their progeny. At the same time it offers a reasonable explanation of the origin and color behavior of green pollen in a cultivated strain of Petunia, and demonstrates the possibility of a single shade or tint of pollen color being represented by two or more genotypes.

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A MATHEMATICAL THEORY OF NATURAL AND ARTIFICIAL SELECTION

PART X. SOME THEOREMS ON ARTIFICIAL SELECTION*

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The former papers of this series have been mainly occupied with cases where no genotype was completely eliminated, though some were fitter than others. The main earlier work dealing with the case where certain genotypes were wholly prevented from breeding, is that of ZIRKLE (1926). He considers selection in random mating populations where the characters are determined by a number of unlinked genes, all present in equal proportions. This case has assumed a special importance in experimental work where a cross is made between pure lines. Thus LITTLE and his colleagues have investigated the susceptibility of mice to tumor transplantation. A tumor can be transplanted into any individual carrying each of k dominant genes, where k varies between 2 and 12 or more (CLOUDMAN 1932). There are only two phenotypes, susceptible and immune. If either of these is bred from in any generation to the exclusion of the other it is clear that in any generation the ratio of dominant to recessive genes will be the same in all the k loci, apart from differences due to the smallness of the sample. This is so however the population is derived, whether it is an F_2, a backcross or some later derivative. In consequence, as ZIRKLE pointed out, we can fix our attention on one of the k gene pairs, and any statement made about it is true for each other pair.

HALDANE (1926) has already dealt with the case where all the genes concerned are not present in equal numbers. This case, though important for evolution and eugenics, is relatively intractable. ZIRKLE'S analysis, though accurate and valuable, can be enormously simplified. Further he confines himself to random mating populations, and a geneticist desiring to fix a character would probably employ inbreeding. Hence certain theorems on inbreeding combined with selection will be proved. Again there is a close analogy between the populations considered and autopolyploid populations.

In each case we shall consider a population derived from an F_1 obtained by mating two pure lines, though many results can be extended to other populations. These latter are marked by an asterisk. The results are

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given for very large populations, but they may be regarded as giving probabilities for smaller populations, provided that these are not so small as to entail appreciable inbreeding where mating is supposed to be at random. We shall suppose that all genes are autosomal and unlinked. By the expression "multiple dominant" is meant a zygote containing at least one of each of the k dominant genes, by "multiple recessive" a zygote containing none of them.

SELECTION OF MULTIPLE DOMINANTS; RANDOM MATING

This case is very simple for the following reason. A zygote is eliminated if it is recessive for any gene. The probability of this being the case is the same whatever other genes are present. Each of the k genes A, B, C,— must be present in a multiple dominant. Since mating is at random the genotypes in F_n are in the ratios

$$u_n^2 AA : 2u_n Aa : 1aa$$

and so for the other genes. The effect of eliminating all recessives for any gene will be to remove all aa zygotes, and some of the AA and Aa. These latter are eliminated in equal proportions, as A is not linked with any of the other genes concerned. The survivors are in the ratio

$$u_n AA : 2Aa,$$

giving gametes in the ratio

$$(u_n+1)A : 1a$$

whence

$$u_{n+1} = u_n + 1$$

just as when one gene only is concerned, and

$$u_n = u_0 + n$$

so that the proportion of multiple dominants in F_n is

$$D_n = \left[1 - \frac{1}{(u_0+n+1)^2}\right]^k. \qquad (1.1^*)$$

But in F_2, $u_2 = 1$, whence $u_n = n-1$, and

$$D_n = (1-n^{-2})^k. \qquad (1.2)$$

This is equivalent to ZIRKLE's formula of p. 562 but simpler. For example if $k=8$, $D_2 = (1-\frac{1}{4})^8$ or .1001, $D_5 = (1-1/25)^8$ or .7218.

RELATION BETWEEN GAMETES OF SUCCESSIVE GENERATIONS OF A RANDOM MATING POPULATION, WITHOUT SELECTION

Before solving the next two problems an expression must be found for this relationship. Let F_n be formed from gametes such that the proportion

of *each* type of gamete carrying r dominants is g_r. There are ${}_kC_r$ types of gamete carrying r dominants, each present in equal numbers. Hence

$$\sum_{r=0}^{k} {}_kC_r g_r = 1.$$

Let Z(i, j) be the proportion in F_n of *each* type of zygote homozygous for i dominant and j recessive genes. Clearly $k \geqslant i+j \geqslant 0$, and there are

$$\frac{k!}{i!j!(k-i-j)!} \text{ or } {}_kC_i\,{}_{k-i}C_j$$

different types of such zygote, so that

$$\sum_{i=0}^{k} {}_kC_i \sum_{j=0}^{k-i} {}_{k-i}C_j\, Z(i, j) = 1.$$

It also follows that such a zygote can be made up in ${}_{k-i-j}C_r$ ways from gametes carrying $(i+r)$ and $(k-j-r)$ dominants, so that

$$Z(i, j) = \sum_{r=0}^{k-i-j} {}_{k-i-j}C_r g_{i+r} g_{k-j-r}. \qquad (2.1^*)$$

Such a zygote produces gametes containing from i to $k-j$ (inclusive) dominant genes in proportions which are terms in the expansion of $\left(\frac{1+1}{2}\right)^{k-i-j}$.

That is to say it contributes to one type of gamete carrying m dominants a proportion $2^{i+j-k}\,{}_{k-i-j}C_{m-i}$ of its gametes. So if $g_m{}^1$ be the proportion of each type of gamete carrying m dominants produced by F_n, we have, since there are ${}_kC_i\,{}_{k-i}C_j$ types of zygote in the proportion Z(i, j) and ${}_kC_m$ types of gamete in the proportion $g_m{}^1$,

$$\begin{aligned} g_m{}^1 &= \frac{1}{{}_kC_m} \sum_{i=0}^{m} \sum_{j=0}^{k-m} 2^{i+j-k}\,{}_{k-i-j}C_{m-i}\,{}_kC_i\,{}_{k-i}C_j\, Z(i, j) \\ &= 2^{-k} \sum_{i=0}^{m} 2^i\,{}_mC_i \sum_{j=0}^{k-m} 2^j\,{}_{k-m}C_j\, Z(i, j) \qquad (2.2^*) \\ &= 2^{-k} \sum_{i=0}^{m} 2^i\,{}_mC_i \sum_{j=0}^{k-m} 2^j\,{}_{k-m}C_j \sum_{r=0}^{k-i-j} {}_{k-i-j}C_r g_{i+r} g_{k-j-r}. \end{aligned}$$

This expression can readily be altered if any classes of zygote are removed by selection.

ELIMINATION OF MULTIPLE DOMINANTS, RANDOM MATING

The classes of zygotes eliminated are recessive for none of the genes, that is to say in the expression for Z(i, j), $j=0$. Hence the proportion of dominants in F_n is

$$D_n = \sum_{i=0}^{k} {}_kC_i \, Z(i, 0) \qquad (3.1^*)$$

and the gametes of the survivors, which form F_{n+1}, are given by the expression

$$g_m{}^1 = \frac{2^{-k} \sum_{i=0}^{m} 2^i \, {}_mC_i \sum_{j=1}^{k-m} 2j \, {}_{k-m}C_i \, Z(i, j)}{1 - D_n} . \qquad (3.2^*)$$

Further in the gametes of F_1, every $g_r = 2^{-k}$, and in all later generations $g_k = 0$.

For purposes of calculation it is desirable to write out the necessary formulae for each Z(i, j) from equation (2.1). These number $\frac{1}{2}(k+1)(k+2)$. They can then be substituted in equations (3.1) and (3.2). For example if $k = 6$ we obtain 28 such expressions as

$$Z(2, 0) = 2g_2g_6 + 8g_3g_5 + 6g_4{}^2 .$$

Whence

$$D_n = 2g_6 - g_6{}^2 + 6g_5(2g_1 + 10g_2 + 20g_3 + 20g_4 + 5g_5) + 30g_4(g_2 + 4g_3 + 3g_4) + 20g_3{}^2$$

$$g_0{}^1 = \frac{1}{8(1-D_n)} [g_0(8g_0 + 48g_1 + 60g_2 + 40g_3 + 15g_4 + 3g_5) + 15g_1(4g_1 + 8g_2 + 4g_3 + g_4) + 15g_2(3g_2 + 2g_3)]$$

$$g_1{}^1 = \frac{1}{16(1-D_n)} [g_0(16g_1 + 40g_2 + 40g_3 + 20g_4 + 5g_5) + g_1(56g_1 + 200g_2 + 160g_3 + 65g_4 + 10g_5) + 10g_2(14g_2 + 17g_3 + 4g_4) + 30g_3{}^2]$$

$$g_2{}^1 = \frac{1}{4(1-D_n)} [g_0(2g_2 + 4g_3 + 3g_4 + g_5) + g_1(2g_1 + 20g_2 + 28g_3 + 17g_4 + 4g_5) + g_2(29g_2 + 62g_3 + 28g_4 + 4g_5) + 12g_3(2g_3 + g_4)]$$

$$g_3{}^1 = \frac{1}{16(1-D_n)} [g_0(4g_3 + 6g_4 + 3g_5) + 3g_1(4g_2 + 16g_5 + 17g_4 + 6g_5) + 6g_2(7g_2 + 31g_3 + 24g_4 + 6g_5) + 2g_3(71g_3 + 78g_4 + 12g_5) + 24g_4{}^2]$$

$$g_4{}^1 = \frac{1}{8(1-D_n)} [g_0(g_4 + g_5) + g_1(4g_3 + 13g_4 + 8g_5) + g_2(3g_2 + 34g_3 + 56g_4 + 24g_5) + 8g_3(6g_3 + 13g_4 + 4g_5) + 8g_4(5g_4 + 2g_5)]$$

$$g_5{}^1 = \frac{1}{16(1-D_n)} [g_0g_5 + 5g_1(g_4 + 2g_5) + 10g_2(g_3 + 4g_4 + 4g_5) + 10g_3(3g_3 + 12g_4 + 8g_5) + 80g_4(g_4 + g_5) + 16g_5{}^2] .$$

Hence $D_2=\frac{729}{4096}=.1780$, and, among the gametes forming F_3

$$g_0=\frac{63}{3367},\quad g_1=\frac{62}{3367},\quad g_2=\frac{60}{3367},\quad g_3=\frac{56}{3367},\quad g_4=\frac{48}{3367},\quad g_5=\frac{32}{3367},\quad g_6=0$$

whence $D_3=\frac{1,416,512}{11,336,689}=.1249$, and D_4 and subsequent terms can easily be calculated.

ZIRKLE has given the requisite expressions for $k=2, 3, 4$, and carried out the calculations. In the case of $k=2$ the composition of each generation may be represented by a single parameter. BENNETT (1924) has solved the equation

$$S_{n+1}=S_n+1-\frac{1}{S_n}, \tag{3.3}$$

which arises if we represent the gametes forming F_n by

$$\frac{1}{S_n+1}Ab,\quad \frac{1}{S_n+1}aB,\quad \frac{S_n-1}{S_n+1}ab,$$

(where $S_3=5/2$).

Here

$$D_n=\frac{2}{(S_n+1)^2},$$

and

$$n+C=S_n+\log(S_n-1)+\frac{1}{2S_n}-\frac{1}{3S_n^2}-\frac{5}{36S_n^3}-\frac{13}{240S_n^4}-\frac{193}{1800S_n^5}-\frac{947}{7560S_n^6}-\cdots\cdots \tag{3.4}$$

where $C=.64018855$ —. An alternative solution is as follows:—

Let the gametes forming F_n be:—

$$\frac{x_n-1}{2x_n}Ab,\quad \frac{x_n-1}{2x_n}aB,\quad \frac{1}{x_n}ab.$$

Then

$$D_n=\frac{(x_n-1)^2}{2x_n^2},\qquad x_{n+1}=\frac{x_n}{2}+1-\frac{1}{2x_n}$$

or

$$\Delta x_n=x_{n+1}-x_n=-\frac{(x_n-1)^2}{2x_n}.$$

This is a particular case of the equation $\Delta x_n=\frac{-k(x_n-1)^2}{x_n}$ solved by

HALDANE (1932) in another connection. It was shown that for $x > 1$, as in this case, a very approximate solution is

$$n = C + \frac{1}{k(x_n - 1)} + \frac{\log x_n}{\log (1-k)} + \frac{1-k}{k} \log_e \left(\frac{x_n}{x_n - 1} \right).$$

Here $k = \frac{1}{2}$, so

$$n = C + \frac{2}{x_n - 1} - \frac{\log x_n}{\log 2} + \log_e \left(\frac{x_n}{x_n - 1} \right)$$

$$= C^1 + \sqrt{\frac{2}{D_n}} + \frac{\log (1 - \sqrt{2D_n})}{\log 2} - \frac{1}{2} \log_e D_n. \qquad (3.5^*)$$

It was shown that this equation has an error of about 1 percent in the neighbourhood of $x_n = 2$, and is much more accurate for smaller values. It may be used to solve such problems as the following: "How many generations of selection are needed to reduce the proportion of double dominants to 1 percent?" In F_3, the first generation for which the equation (3.5) holds, we substitute $n = 3$, $D_n = 2/9$, and find $C^1 = .833$. Substituting this value, and $D_n = .01$, we find $n = 12.005$. Hence the value is very nearly reached in F_{12}.

ELIMINATION OF A MULTIPLE RECESSIVE, RANDOM MATING

Under any type of mating, selection of a multiple recessive is of course complete in one generation. To derive the equations for the case where the single genotype recessive for all k genes is eliminated we have only to note that the proportion of multiple recessives $Z(0, k)$ of equation (2.2) is $R_n = g_0^2$ and the relation between gametes of successive generations is

$$(1 - R_n) g_m^1 = 2^{-k} \sum_{i=0}^{m} 2^i \, {}_mC_i \sum_{j=0}^{k-m} 2^j \, {}_{k-m}C_j \, Z(i, j), \text{ when } m \neq 0, \text{ and}$$

$$(1 - R_n) g_0^1 = 2^{-k} \sum_{j=0}^{k-1} 2^j \, {}_kC_j \, Z(0, j). \qquad (4.1^*)$$

ZIRKLE has tabulated values of R_n for $k = 2$, 3, and 4; and higher values of k are not known to occur. If $k = 2$, and $p_n = g_2$, $q_n = g_1$, $r_n = g_0$ we have, as he points out

$$p_{n+1} = \frac{p_n + \frac{1}{2}(q_n^2 - p_n r_n)}{1 - r_n^2}$$

$$q_{n+1} = \frac{q_n - \frac{1}{2}(q_n^2 - p_n r_n)}{1 - r_n^2}$$

$$r_{n+1} = \frac{r_n - r_n^2 + \frac{1}{2}(q_n^2 - p_n r_n)}{1 - r_n^2}.$$

Putting $q_n + r_n = z_n$, we find

$$z_{n+1} = \frac{z_n - r_n^2}{1 - r_n^2}$$

$$r_{n+1} = \frac{\frac{1}{2}(r_n + z_n^2) - r_n^2}{1 - r_n^2} \qquad 4.2^*)$$

$$R_{n+1} = r_n^2.$$

Whence calculation is easy, since $z_2 = \frac{1}{2}$, $r_2 = \frac{1}{4}$. For large values of n, r_n approximates to z_n^2, and Δz_n to $-z_n^4$, whence $n = \text{constant} + \frac{1}{3} z^{-3}$, approximately, and R_n approximates to $(3n + c)^{-4/3}$.

SELECTION OF DOMINANTS IN AN AUTOTETRAPLOID, RANDOM MATING

This case is included here owing to its close similarity to the last. It is assumed that double reduction (HALDANE 1930) does not occur. Let the gametes forming F_n be in the ratios $p_n AA : 2q_n Aa : r_n aa$, where $p_n + 2q_n + r_n = 1$. The recessives, which are eliminated, occur in F_n in the proportion $R_n = r_n^2$, and:—

$$p_{n+1} = \frac{p_n + \frac{2}{3}(q_n^2 - p_n r_n)}{1 - r_n^2}$$

$$q_{n+1} = \frac{q_n - \frac{2}{3}(q_n^2 - p_n r_n)}{1 - r_n^2}$$

$$r_{n+1} = \frac{r_n - r_n^2 + \frac{2}{3}(q_n^2 - p_n r_n)}{1 - r_n^2}.$$

Putting $z_n = q_n + r_n$, we have

$$z_{n+1} = \frac{z_n - r_n^2}{1 - r_n^2}$$

$$r_{n+1} = \frac{\frac{1}{3}(r_n + 2z_n^2) - r_n^2}{1 - r_n^2} \qquad (5.1^*)$$

$$R_n = r_n^2.$$

As above, when n is large, R_n approximates to $(3n + c)^{-4/3}$. Starting with an F_1 between homozygotes, we find the following percentage values of R_n in successive generations:—0, 2.7, 4.0, 3.671, 6.432, 2.623, 2.003, 1.644. . . . It will be seen that the values oscillate at first, as they do when there is no selection, reaching a maximum in F_5.

SELECTION OF MULTIPLE DOMINANTS: SELF-FERTILIZATION

A self-fertilized population consists of lines of one individual per generation. Selection does not alter the character of these lines, but only their

relative proportions. If there is no selection F_{n-1} consists of zygotes in the ratios $(2^{n-1}-2)\ AA:4\ Aa:(2^{n-1}-2)aa$, and similarly for the other genes. Now (if we confine our attention to A and a) all the aa zygotes are eliminated. But as all their descendants in later generations are also aa, the composition of F_n is unaltered if we postpone our selection till F_{n-1}. The parents of F_n, after selection, are thus in the ratio

$$(2^{n-1}-2)AA:4Aa,$$

and F_n consists of $(2^{n-1}-1)AA:2Aa:1aa$ or $\dfrac{2^{n-1}+1}{2^{n-1}+2}\ AA$ and Aa. Hence the proportion of multiple dominants in F_n is

$$D_n=\left(1-\frac{1}{2^{n-1}+2}\right)^k. \tag{5.1}$$

Comparing this with $(1-n^{-2})^k$ of equation (1.2) we see that from F_3 to F_6 inclusive the multiple dominants are fewer than in the case of random mating, but from F_7 onwards more numerous. This is because the rapid elimination of heterozygotes causes a large number of recessives to appear in the first few generations. The final population consists of homozygous multiple dominants.

ELIMINATION OF MULTIPLE DOMINANTS; SELF-FERTILIZATION

This case is extremely simple. Elimination is complete in one generation. For clearly no multiple dominant can appear save in the progeny of a zygote carrying all the requisite genes, that is, itself a multiple dominant. But the end result is quite different from that in the case of random mating. In the latter case all dominant genes are ultimately eliminated in a large population, and most in a small one. With self-fertilization no selective elimination occurs after F_2, and the average number of genes for which a homozygote in the final population is dominant is $\dfrac{2k(4^{k-1}-3^{k-1})}{4^k-3^k}$, or $\dfrac{k}{2}\left(1-\dfrac{3^{k-1}}{4^k-3^k}\right)$. The multiple dominant phenotype will thus reappear if crossbreeding commences in this heterogeneous population.

ELIMINATION OF MULTIPLE RECESSIVES; SELF-FERTILIZATION

This case is of some practical importance in allopolyploid plants such as wheat. As in the last case but one we can imagine selection postponed either until F_{n-1} or F_n without altering the final result. The complete P_{n-1} would have consisted of

$$(\tfrac{1}{2}-2^{1-n})AA,\ 2^{2-n}Aa,\ (\tfrac{1}{2}-2^{1-n})aa,$$

and so on for the other $k-1$ genes, the total multiple recessives removed up to F_{n-1} being therefore $(\frac{1}{2}-2^{1-nk})$. The additional multiple recessives removed from F_n would therefore be $(\frac{1}{2}-2^{-n})^k-(\frac{1}{2}-2^{1-n})^k$ taken from a population of $1-(\frac{1}{2}-2^{1-n})^k$ of the original total. Thus the proportion of recessives appearing in F_n is

$$R_n=\frac{(\frac{1}{2}-2^{-n})^k-(\frac{1}{2}-2^{1-n})^k}{1-(\frac{1}{2}-2^{1-n})^k}$$
$$=\frac{(2^{n-1}-1)^k-(2^{n-1}-2)^k}{2^{nk}-(2^{n-1}-2)^k}. \qquad (6.1)$$

For large values of n this approximates to $2^{1-n-k}k$. Table 1 gives the value of $100\ R_n$, the percentage of multiple recessives, for F_2 to F_{10} for $k=2$ and 3, the practically important cases.

TABLE 1

Percentages of multiple recessives in F_n, recessives eliminated by selfing.

n	2	3	4	5	6	7
k=2, 100 R_n	6.25	8.3	5.90	3.502	1.909	0.9968
k=3, 100 R_n	1.5625	3.770	3.273	2.102	1.187	0.6374

n	8	9	10
k=2, 100 R_n	0.5095	0.2435	0.1295
k=3, 100 R_n	0.3112	0.1649	0.08308

As in the last case, selection does not lead to a homogeneous population. It ceases in any line as soon as any single recessive gene is eliminated. The average number of dominant genes in a homozygote of the final population is $\frac{k}{2-2^{1-k}}$, instead of k in the case of random mating. But of course the elimination of the multiple recessives is greatly speeded up by selfing.

SELECTION OF DOMINANTS IN AN AUTOTETRAPLOID: SELF-FERTILIZATION

HALDANE (1930) has shown that in a self-fertilized autotetraploid F_n, where F_1 is a hybrid between two homozygotes, the proportion of recessives is

$$r_n=\frac{1}{2}\left[1-\frac{7}{5}\left(\frac{5}{6}\right)^n+\left(\frac{1}{6}\right)^n\right].$$

Hence, as above, the proportion appearing in F_n when recessives are eliminated in each generation is

$$R_n = \frac{r_n - r_{n-1}}{1 - r_{n-1}}$$

Hence

$$R_n = \frac{5(7.5^{n-3} - 1)}{6(6^{n-1} + 7.5^{n-3} - 1)} \qquad (7.1)$$

and when n is very large it approximates to $\frac{7.5^{n-2}}{6^n}$ or $\frac{1}{6}$ of the total proportion of heterozygotes in an unselected F_{n-1}.

SELECTION OF A SINGLE DOMINANT, BROTHER-SISTER MATING

Before we can proceed to consider the selection of a multiple dominant, this problem, which so far as I know has never been fully treated, must be solved. Since *aa* zygotes are not allowed to breed, there are only three types of mating. Let these occur among the parents of F_n in the proportions $x_n AA \times AA$, $y_n AA \times Aa$ (and reciprocally) and $z_n Aa \times Aa$, where $x_n + y_n + z_n = 1$. Then the proportion of dominants in F_n is $d_n = 1 - \frac{1}{4}z_n$. Only 3/4 of the offspring of the $Aa \times Aa$ matings are allowed to breed. Hence the contribution of these matings to the next generation is

$$\frac{3}{4}\left(\frac{1}{9}AA \times AA + \frac{4}{9}AA \times Aa + \frac{4}{9}Aa \times Aa\right)$$

or

$$\frac{1}{12}AA \times AA,\ \frac{1}{3}AA \times Aa,\ \frac{1}{3}Aa \times Aa.$$

Hence:—

$$x_{n+1} = \frac{x_n + \frac{1}{4}y_n + \frac{1}{12}z_n}{d_n}$$

$$y_{n+1} = \frac{\frac{1}{2}y_n + \frac{1}{3}z_n}{d_n} \qquad (8.1^*)$$

$$z_{n+1} = \frac{\frac{1}{4}y_n + \frac{1}{3}z_n}{d_n}.$$

Putting $z_n = 4 - 4d_n$ we find

$$y_{n+1} = \frac{\frac{1}{2}y_n + \frac{4}{3} - \frac{4}{3}d_n}{d_n}$$

and

$$4-4d_{n+1}=\frac{\frac{1}{4}y_n+\frac{4}{3}-\frac{4}{3}d_n}{d_n}$$

Hence

$$\frac{3}{4}y_{n+1}=\frac{\frac{3}{8}y_n+1}{d_n}-1$$

and

$$\tfrac{3}{4}y_n=16d_n-12d_{n+1}d_n-4$$

so that

$$12d_{n+2}d_{n+1}d_n-22d_{n+1}d_n+11d_n-1=0 \qquad (8.2^*)$$

If $d_n=\frac{p_{n+1}}{p_n}$, we find

$$12p_{n+3}-22p_{n+2}+11p_{n+1}-p_n=0$$

p_n is therefore the sum of the nth terms of three geometric series whose common ratios are the roots of $12\lambda^3-22\lambda^2+11\lambda-1=0$, or 1 and $\frac{1}{5\pm\sqrt{13}}$.

Since $z_2=1$, we find

$$p_{n+2}=2+\frac{1+\frac{1}{\sqrt{13}}}{(5-\sqrt{13})^n}+\frac{1-\frac{1}{\sqrt{13}}}{(5+\sqrt{13})^n} \qquad (8.3)$$

If r_n and h_n are the proportions of recessives and heterozygotes respectively in F_n, then $r_n=1-d_n$, so from equation (8.2)

$$r_{n+2}=\frac{1+10r_{n+1}-\frac{1}{1-r_n}}{12(1-r_{n+1})} \qquad (8.4^*)$$

from which r_n is easily calculated, since $r_2=\frac{1}{4}$, $r_3=\frac{1}{9}$. Since $r_n=\frac{p_n-p_{n+1}}{p_n}$, then from equation (8.3),

$$r_{n+3}=\frac{\left(2-\frac{7}{\sqrt{13}}\right)(5-\sqrt{13})^{-n}+\left(2+\frac{7}{\sqrt{13}}\right)(5+\sqrt{13})^{-n}}{2+\left(1+\frac{1}{\sqrt{13}}\right)(5-\sqrt{13})^{-2}+\left(1-\frac{1}{\sqrt{13}}\right)(5+\sqrt{13})^{-n}} \qquad (8.5)$$

Hence the values of r_n ultimately approximate to $\left(1-\frac{7}{2\sqrt{13}}\right)(5-\sqrt{13})^{3-n}$, a geometric series whose common ratio is .717, thus diminishing more rapidly than in the case of random mating.

$$\begin{aligned} h_n &= \tfrac{1}{2}(y_n + z_n) \\ &= \tfrac{2}{3}(13d_n - 12d_{n+1}d_n - 1) \\ 8 &= r_{n+1}(1 - r_n) - \tfrac{2}{3}r_n \end{aligned} \qquad (8.6^*)$$

whence it is readily calculated. When n is large the value of $\frac{h_n}{r_n}$ approximates to $\frac{8+2\sqrt{13}}{3}$, or 5.07. Hence h_n approximates to a geometric series whose common ratio is .717, and it diminishes more rapidly than in the case of brother-sister mating without selection, where the common ratio is $\frac{1}{\sqrt{5}-1}$, or .809. Table 2 gives the values of r_n and h_n for the first 12 generations, calculated from equations (8.4) and (8.6). They are compared with the corresponding values in the case of random mating. It will be seen that after F_3 the proportion of homozygous dominants is increased by inbreeding, that of heterozygotes diminished. However the proportion of recessives between F_4 and F_{11} inclusive is higher when inbreeding is practiced than when mating is at random. It may be remarked that there is no advantage in beginning brother-sister mating before F_3, since the F_2 from two pure lines are all as closely related to one another genetically as if they were sibs.

In practice the elimination of recessive genes could of course be increased still further either by a refusal to breed from individuals with recessive sibs, or better, by test matings with recessives. But the consideration of this somewhat artificial case is necessary if we are to solve the next problem.

TABLE 2

n	RANDOM MATING		BROTHER-SISTER MATING	
	RECESSIVES	HETEROZYGOTES	RECESSIVES (r_n)	HETEROZYGOTES (h_n)
1	0	1.00	0	1.00
2	.25	.50	.25	.50
3	.1	.4	.1	.4
4	.0625	.375	.072916	.35416
5	.0400	.3200	.054307	.27341
6	.036	.27	.040924	.20726
7	.020408	.2449	.030569	.15497
8	.015625	21875	.022609	.11464
9	.012346	.19753	.016555	.084109
10	.0100	.1800	.012097	.061335
11	.008265	.165289	.0087854	.045115
12	.00694	.1527	.0063392	.032127

SELECTION OF MULTIPLE DOMINANTS, BROTHER-SISTER MATING

The situation is very similar to that in the case of random mating. All the zygotes recessive for any one of the k genes are eliminated at once in each generation. The process of the last paragraph thus takes place for each gene separately. And these processes are independent. So to find the proportion of multiple dominants in F_n we have only to put

$$D_n = (1-r_n)^k \qquad (9.1^*)$$

where r_n is given by equation (8.5) or table 2. Thus in F_7 of an inbred population a fraction .96943 carry any given one of the dominant genes, and $.96943^k$ carry all of them. A fraction $1-h_n-r_n$ or .81446 of F_7 is homozygous for any one dominant gene, a fraction $(1-h_n-r_n)^k$ or $.81446^k$ for all of them. Table 3 gives the results for $k=10$, with random mating results for comparison. It will be seen that up to F_{12} inbreeding slightly slows down the actual appearance of dominants, but greatly increases their genetic purity. Thus in F_{10} 53 percent of the dominants are homozygous in the case of inbreeding, and only 13 percent in that of random mating.

Again there is no need to practice brother-sister mating before F_3, and it would be practicable to begin it in F_2 even if as many as 10 genes were concerned. The process of selection could of course be speeded up if families containing any recessives were rejected, which would be practicable after about F_5.

TABLE 3

Composition of F_n when only multiple dominants for 10 genes are bred from.

n	RANDOM MATING		BROTHER-SISTER MATING	
	TOTAL 10-ple DOMINANTS	HOMOZYGOUS 10-ple DOMINANTS	TOTAL 10-ple DOMINANTS	HOMOZYGOUS 10-ple DOMINANTS
1	1.0	0	1.0	0
2	.0563	9.5×10^{-7}	.0563	9.5×10^{-7}
3	.3080	.00030	.3080	.00030
4	.5244	.00317	.4690	.00381
5	.6648	.01152	.5722	.01886
6	.7720	.02578	.6585	.05769
7	.8137	.04582	.7331	.1284
8	.8543	.06921	.7956	.2285
9	.8832	.09483	.8453	.3460
10	.9043	.1215	.8854	.4665
11	.9203	.1486	.9155	.5746
12	.9327	.1755	.9385	.6754

ELIMINATION OF MULTIPLE DOMINANTS: BROTHER-SISTER MATING

The population in F_2 and later consists of pairs of mated zygotes, apart from the multiple dominants, which are eliminated. Thus mating pairs fall into four classes.

1. Pairs giving only multiple dominants.
2. Pairs giving multiple dominants and other genotypes among their immediate offspring, but no multiple dominants later.
3. Pairs giving multiple dominants and other genotypes both among their immediate progeny and in later generations.
4. Pairs never giving multiple dominants.

Clearly it is only class 3 which contribute multiple dominants to remote generations.

A consideration of the case when $k=2$ will illustrate the principles involved. Nine-sixteenths of F_2 are double dominants, so $D_2=9/16$. The five genotypes which are the parents of F_3 occur in the proportions:

$$\frac{1}{7}AAbb,\ \frac{1}{7}aaBB,\ \frac{2}{7}Aabb,\ \frac{2}{7}aaBb,\ \frac{1}{7}aabb.$$

The matings occur with the following frequencies:—

Class 1. 2/49 $AAbb\times aaBB$

Class 2. 4/49 $AAbb\times aaBb$, 4/49 $aaBB\times Aabb$

Class 3. 8/49 $Aabb\times aaBb$

Class 4. 31/49 other matings, for example, 1/49 $AAbb\times AAbb$, 4/49 $Aabb\times aabb$.

That is to say 2/49 give all double dominants, 8/49 give 50 percent, and 8/49 give 25 percent. So $D_3=8/49$, and 41/49 of F_3 is available for mating. Only class 3 matings give further double dominants. A mating of class 3 gives 25 percent double dominants, and matings which can be symbolized by

$$\frac{3}{4}\cdot\frac{1}{9}(Aabb+aaBb+aabb)^2.$$

If there is a proportion p_n of such matings among the parents of F_n, then $D_n=\frac{1}{4}p_n$,
and

$$p_{n+1}=\frac{\frac{1}{8}p_n}{1-\frac{1}{4}p_n}.$$

If we put

$$\frac{x_{n+1}}{x_n}=1-\frac{1}{4}p_n$$

we find

$$6x_{n+2}-7x_{n+1}+x_n=0,$$

so that

$$x_n=a+6^{-n}$$

Since 6/41 of the matings of the parents of F_4 are derived from families of the type $(Aabb+aaBb+aabb)$,

$$p_4=\frac{2}{9}\cdot\frac{6}{41}=\frac{4}{123}, \text{ and } \frac{x_5}{x_4}=\frac{122}{123}.$$

Hence $a=609\times6^{-5}$ and

$$\frac{x_{n+1}}{x_n}=\frac{1+609\times6^{n-4}}{6+609\times6^{n-4}}.$$

So

$$D_n=1-\frac{x_{n+1}}{x_n}=\frac{5}{6+609\times6^{n-5}} \qquad (10.1)$$

from F_4 onwards. Hence the successive values of D_n, the proportion of double dominants in successive generations from F_2 onwards are $\frac{9}{16}$, $\frac{8}{49}$, $\frac{1}{123}$, $\frac{1}{732}$, $\frac{1}{3186}$ and so on. The corresponding values for a random mating population, derived from equation (3.3), are $\frac{9}{16}$, $\frac{8}{49}$, $\frac{200}{1681}$, $\frac{192,200}{2,193,361}$, .0658, et cetera which diminish far more slowly.

The final population can readily be calculated. Such a mating as $Aabb\times aabb$ ultimately gives a population of 1/4 $AAbb$, 3/4 $aabb$ and so on. The mating $Aabb\times aaBb$ gives 1/6 similar matings and 7/12 matings ultimately leading to

$$\frac{1}{7}AAbb,\ \frac{1}{7}aaBB,\ \frac{5}{7}aabb$$

So the final population is

$$\frac{1}{12}\left(1+\frac{1}{6}+\frac{1}{6^2}+\cdots\right)(AAbb+aaBB+5aabb)$$

or 1/10 $(AAbb+aaBB+5aabb)$. The ultimate population from all the F_2 mating types is therefore in the ratios 54 $AAbb$:54 $aaBB$:95 $aabb$.

In general the proportion of class 4 matings, which never give any multiple dominants, among the survivors of F_2 can easily be shown to be $\frac{16^k-15^k}{(4^k-3^k)^2}$ a quantity which rises from 63.3 percent when $k=2$ to 86.6 per-

cent when $k=10$. In all cases a majority of the matings of F_2 never give multiple dominants, and thus if a few lines are started, most of them will give the desired phenotype only, though they will give different genotypes. There is thus no practical value in working out expressions for D_n for different values of k.

ELIMINATION OF MULTIPLE RECESSIVES; BROTHER-SISTER MATING

The principles to be employed are illustrated when $k=2$. The mating population consists of 8 genotypes other than *aabb*. Let the matings among the survivors of F_n occur in the following proportions:

c	$AABB\times AABB$	g	$AABb\times Aabb^*$
	$AABB\times AABb^*$		$AaBb\times AAbb^*$
	$AABB\times AAbb^*$	h	$AABb\times aaBb^*$
	$AABb\times AABb$	i	$AAbb\times aaBB$
	$AABb\times AAbb^*$	j	$AAbb\times Aabb^*$
	$AAbb\times AAbb^*$	k	$AAbb\times aaBb^*$
d	$AABB\times AaBb$	l	$AaBb\times AaBb$
	$AABb\times AaBB$	m	$AaBb\times Aabb^*$
e	$AABB\times Aabb^*$	p	$Aabb\times Aabb^*$
	$AABb\times aaBB^*$	q	$Aabb\times aaBb$
f	$AABb\times AaBb^*$		

Here reciprocals are included together. The symbol c denotes c_n, c′ denotes c_{n+1}, and so on. An asterisk denotes that there is another similar mating obtained by substituting *A* for *B*. The various matings whose total frequency is c never produce double recessives. The number of these,

$$R'=\frac{1}{16}l_n+\frac{1}{8}m_n+\frac{1}{4}p_n+\frac{1}{4}q_n. \qquad (10.1^*)$$

The remaining mating types (d to k) give no double recessives in the first generation, but do so later. Also $l_1=1$. Hence

$$(1-R')c'=c_n+\frac{7}{16}d_n+\frac{1}{4}e_n+\frac{19}{64}f_n+\frac{1}{4}g_n+\frac{1}{16}h_n+\frac{1}{4}j_n+\frac{31}{240}l_n+\frac{1}{14}m_n+\frac{1}{12}p_n$$

$$(1-R')d'=\frac{1}{4}d_n+\frac{1}{8}f_n+\frac{1}{15}j_n$$

$$(1-R')e'=\frac{1}{16}f_n+\frac{1}{15}l_n$$

$$(1-R')f'=\frac{1}{4}d_n+\frac{1}{2}2_n+\frac{3}{16}f_n+\frac{1}{8}g_n+\frac{1}{4}h_n+\frac{2}{15}l_n+\frac{1}{14}m_n$$

$$(1-R')g'=\frac{1}{8}f_n+\frac{1}{4}g_n+\frac{2}{15}l_n+\frac{1}{7}m_n$$

$$(1-R')h'=\frac{1}{32}f_n+\frac{1}{8}h_n+\frac{1}{15}l_n+\frac{1}{28}m_n$$

$$(1-R')i'=\frac{1}{120}l_n$$

$$
\begin{aligned}
(1-R')j' &= \tfrac{1}{8}f_n+\tfrac{1}{8}g_n+ && \tfrac{1}{2}j_n+\tfrac{1}{30}l_n+\tfrac{1}{14}m_n+\tfrac{1}{3}p_n\\
(1-R')k' &= && \tfrac{1}{30}l_n+\tfrac{1}{28}m_n\\
(1-R')l' &= \tfrac{1}{12}d_n+\tfrac{1}{4}e_n+\tfrac{1}{16}f_n+\tfrac{1}{16}g_n+\tfrac{1}{4}h_n+\ i_n+ && \tfrac{1}{4}k_n+\tfrac{1}{15}l_n+\tfrac{1}{14}m_n+\ \tfrac{1}{12}q_n\\
(1-R')m' &= \tfrac{1}{16}f_n+\tfrac{1}{8}g_n+\tfrac{1}{4}h_n+ && \tfrac{1}{2}k_n+\tfrac{2}{15}l_n+\tfrac{3}{14}m_n+\ \tfrac{1}{3}q_n\\
(1-R')p' &= \tfrac{1}{64}f_n+\tfrac{1}{16}g_n+\tfrac{1}{16}h_n+ && \tfrac{1}{4}j_n+\tfrac{1}{4}k_n+\tfrac{1}{30}l_n+\tfrac{5}{56}m_n+\tfrac{1}{3}p_n+\tfrac{1}{6}q_n\\
(1-R')q' &= && \tfrac{1}{30}l_n+\tfrac{1}{14}m_n+\ \tfrac{1}{6}q_n
\end{aligned}
\qquad (10.2^*)
$$

Hence

$$R_2=\frac{1}{16},\quad R_3=\frac{1}{25},\quad R_4=\frac{4649}{103680}=4.48 \text{ percent}$$

whereas with random mating (from equation 4.2)

$$R_2=\frac{1}{16},\quad R_3=\frac{1}{25},\quad R_4=\frac{361}{11664}=3.10 \text{ percent}.$$

Thus at first somewhat more double recessives appear as the result of inbreeding. However it is clear that in the case of inbreeding R_n ultimately approximates to a geometrical series, and consequently diminishes far more rapidly than in the case of random mating. For practical purposes the value of c_n is even more important than that of $R_n \cdot c_2=\frac{31}{225}=13.8$ per-cent, while $c_3=\frac{14319}{45360}=31.6$ percent. That is to say 31.6 percent of all F_3 mated pairs will give no double recessives. Hence even two generations of brother-sister mating will have eliminated the possibility of producing double recessives from many lines.

For values of k exceeding 2 the equations become quite excessively complex. However c_2, the probability that a given breeding pair in F_2 (after eliminating the multiple recessives) will never, in any later generation, yield multiple recessives, is

$$c_2=\frac{16^k-15^k}{(4^k-1)^2}. \qquad (10.3)$$

The values of c_2 as percentages from $k=1$ to 5 inclusive are 11.1, 13.7, 18.17, 22.93, 27.63. Thus if $k=5$ rather over 1/4 of all the matings of surviving F_2 would give no recessives. c_2 increases rather slowly, only reaching 47.55 percent when $k=10$, and 72.49 percent when $k=20$.

Just as in the case where multiple dominants are eliminated, the final population is not genetically homogeneous in the case of brother-sister mating. If mating were re-started between different lines, multiple recessives might appear in the second, though not in the first, generation.

The rather delicate problem of brother-sister mating in an autopolyploid is reserved for a future publication.

SUMMARY

Expressions are found for the effects of selection on populations, both random mating, selfed and inbred, where the character selected depends upon several genes, and (as in the case of crosses between pure lines) each gene pair is present in the same ratio.

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CYTOGENETIC STUDIES OF AN INTERCHANGE BETWEEN CHROMOSOMES 8 AND 9 IN MAIZE

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The partially sterile line of maize considered in this paper originated as one of three partially sterile plants in a culture of sixty plants from an ear of red aleurone (*pr*) waxy (*wx*) maize which was being used as a standard normal stock. The stock had not been subjected to X-ray treatment. This partially sterile line of maize has been described in a preliminary note (BURNHAM 1930) as semisterile-2. It was reported then that the "semisterile" plants showed at diakinesis and metaphase I of meiosis a ring of four chromosomes representing an association of two of the smaller pairs. Linkage tests showed that one of these pairs carries the shrunken-waxy linkage group. MCCLINTOCK (1930) has shown that the second and third smallest chromosomes, 9 and 8, are involved; and, by a study of midprophase stages, that there has been an interchange between them of unequal terminal segments of the long arms, chromosome 8 having lost the larger piece. Her trisomic tests showed that chromosome 9 is the one carrying the waxy linkage group. BEADLE (1932) has used this interchange to mark the maize chromosomes 8 and 9 for a cytogenetical study of the hybrid with Euchlaena. CREIGHTON and MCCLINTOCK (1931) have used it as one of the morphological markers in their demonstration that an actual exchange of pieces of homologous chromosomes accompanies genetic crossing over.

The present paper presents additional cytogenetical data on this interchange. In order to have the genetic data together in this paper, Dr. H. B. CREIGHTON has furnished the data in table 3 which she has collected on crossing over with aurea 1 (au_1) and virescent 1 (v_1).

For convenience the symbol T will be used to represent the complex with respect to the chromosomes involved in the interchange. Different interchanges may be differentiated by adding numerals indicating the chromosomes concerned. The one being considered here will be referred to henceforth as T 8–9. The term "X-normal" has been proposed (BRINK and BURNHAM 1929) for plants homozygous for an interchange; while BLAKESLEE uses the term "Prime type" in Datura. In this paper, no special term will be used, reserving T 8–9 for the interchange complex.

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BREEDING BEHAVIOR OF SEMISTERILE PLANTS

Semisterile plants, when used as the pollen parents in crosses with normals, gave, out of 2812 plants, 47.4 percent that were semisterile, the remainder being normal, where a 1:1 ratio was expected. Where semisterile plants were used as the female parents in crosses with normals, out of 8141 plants, 49.7 percent were semisterile. The small deficiency noted in the former is similar in amount to that observed by BRINK (1925) in crosses using plants heterozygous for *wx* as the pollen parents. In these crosses the waxy gene (*wx*) entered with the T 8–9 complex. The cross in which *wx* was in the normal chromosome has not been tested in sufficient numbers for comparison.

The progeny of a self-pollinated semisterile plant includes normals and semisteriles. Among the normals there should be plants homozygous for the interchange. Since the gene *wx* entered with the T 8–9 complex and is rather closely linked with it, the waxy seeds from such a self-pollinated plant should be homozygous for T 8–9 in most cases. This proved to be the case and facilitated their isolation. The plants of this homozygous stock show no external differences from the semisterile sibs or from normal plants. They have ten bivalents at meiosis and show no more ovule or pollen abortion than do normal plants. When crossed with a standard normal line, all the F_1 plants are semisterile. This serves as the genetic test for the isolation of lines homozygous for an interchange. In the earlier report (BURNHAM 1930), the cross of T 8–9 with T 1–2a (semisterile-1) was found to give plants having two separate rings of four at meiosis and about 79 percent pollen abortion. From this cross, a fertile stock homozgous for both interchanges has been isolated. When crossed with standard normals, all the F_1 plants show the high sterility (79 percent).

Semisterility behaves in linkage tests as though it were at one definite locus in each of the two chromosomes involved in the interchange. These loci must correspond to the points at which the breakages and reattachments originally occurred. Tests for linkage of genes with the points of breakage are made by crossing a semisterile plant or one homozygous for the interchange with a normal stock carrying recessive factors. The semisterile F_1 plants (carrying the interchange) are then back-crossed to the same normal recessive stock. The relative numbers of semisterile and normal plants in each of the resulting genetic classes indicate the strength of the linkage. The classifications for sterility were made by examination under the microscope of tassel samples taken from the plants at the time of pollen shedding. In a semisterile plant approximately half of the grains are aborted. It might seem possible to use ear classifications since the ears on plants heterozygous for T 8–9 are only partially filled. In one experiment

TABLE 1

Summary of linkage from backcrosses involving T 8-9 and factors in chromosomes 8 and 9. The three-point and four-point data are included.

GENES	LINKAGE	NUMBER OF INDIVIDUALS					RECOMBINATIONS	
x Y	PHASE	*XY*	*Xy*	*xY*	*xy*	TOTAL	NUMBER	PERCENT
au$_1$* T 8–9	RB	617	43	53	574	1237	96	7.5
c T 8–9 (♂)	CB	116	54	55	126	351	109	31.1
c T 8–9 (♀)	CB	42	37	32	58	169	69	40.8
total						520	178	34.2
sh T 8–9 (♀)	CB	347	183	121	274	925	304	32.9
v$_1$* T 8–9	RB	650	17	21	563	1251	38	3.0
wx T 8–9 (♂)	RB	48	430	433	70	981	118	12.0
wx T 8–9 (♀)	RB	136	974	952	170	2232	306	13.7
wx T 8–9 (♂)	CB	99	23	14	63	199	37	18.6
wx T 8–9 (♀)	CB	103	15	17	61	196	32	16.3
total						3608	493	13.7
j T 8–9 (♂)	CB	53	34	50	33	170	84	49.4
j T 8–9 (♀)	CB	92	43	32	77	244	75	30.7
total						414	159	38.4

* Data furnished by H. B. CREIGHTON.

where pericarp was to be noted later, the plants were tagged and numbered and separate classifications for sterility based on the ears and on the pollen were made. Out of 190 plants, 3 of those having normal pollen had been classed as semisterile on the basis of the appearance of the ears. The errors were all in this one direction. Plants having small ears were not included. In this case no weak recessives were segregating. Where they are, the percentage error might be much higher, since such plants have smaller ears and often silk late when there is little pollen being shed. When using the pollen for classification, certain precautions are also necessary. The most important of these seems to be that certain of the linkage testers in use show pollen abortion which is not associated with ovule abortion. In crosses with standard normals, however, this sterility has not proved to be dominant.

TABLE 2

Three-point linkage data from backcrosses involving T 8–9 and factors in chromosomes 8 and 9.

GENETIC CONSTITUTION	PARENTAL COMBINATIONS		RECOMBINATIONS						TOTAL
			REGION 1		REGION 2		REGIONS 1, 2		
C+T	42	58	2	4	35	28	0	0	169
c sh + (♀)	100		6		63		0		
			3.6%		37.3%				
c++	114	89	45	27	10	14	12	1	312
C wx T (♂)	203		72		24		13		
			27.2%		11.9%				
+ *wx* T	205	171	82	49	40	17	6	3	573
sh ++ (♀)	376		131		57		9		
			24.4%		11.5%				
++T	77	39	13	13	14	13	3	—	172
sh wx + (♀)	116		26		27		3		
			16.9%		17.4%				
++*j*	61	68	12	1	38	23	1	2	206
wx T + (♀)	129		13		61		3		
			7.8%		31.1%				
++*j*	15	33	6	3	20	36	4	5	122
wx T+ (♂)	48		9		56		9		
			14.8%		53.3%				

The new two-point data on the linkages obtained with T 8–9 are given in table 1. The combined data for factors in chromosome 9 indicate: 34.2 percent recombination with *c*, 32.9 percent with *sh*, and 13.7 percent with *wx*. The tests with *wx* show apparently significant deviations from equality in the two recombination classes. In the *Wx* class the data show 11.6 percent recombination while in the *wx* class they show 14.8 percent. The small deficiency of semisterile offspring obtained in crosses of normals with the pollen of semisterile plants would explain this difference, but although no deficiency in number of semisterile offspring was found when semisteriles were used as the female parents, the same difference is found in the percentages of recombination in the *Wx* and *wx* classes. However, the data from individual ears are not consistent in showing this. The cause of the difference, if it exists, is not known at present.

The two-point data, and the three-point data in table 2 show that the break lies on the side of *sh* away from *wx;* that is, that the order is: *c–sh–wx*–T 8–9. The earlier data (BURNHAM 1930) from 2n+1 plants which were partially sterile had indicated this as the position of the break. These data show the order of the genes from the point of interchange but do not determine on which side of this point they lie. MCCLINTOCK (1931), by the use of a $2n+1$ or trisomic type carrying only one of the interchanged chromosomes in T 8–9, showed that these genes lie in the part of chromosome 9 possessing the spindle fiber insertion region and not in the piece translocated to chromosome 8. (The term "interchanged" or "translocated piece" is used to designate the piece which does not include the fiber insertion region). This conclusion is also substantiated by my data on a 5–9 interchange (BURNHAM 1934) in which the break in chromosome 9 occurred in the short arm.

The data furnished by CREIGHTON in table 3 show 3 percent of recombination between T 8–9 and v_1, and 7.5 percent between T 8–9 and au_1. These 4-point data also indicate that au_1 and v_1 are between *wx* and the break, with v_1 closer to the break than au_1.

Data from another interchange, T 5–9, show that the genes *sh* and *wx* are in the short arm of chromosome 9 (BURNHAM 1934). From the genetic data, it seems probable that v_1 is in the long arm of that chromosome. Cytologically, in T 8–9 the break in chromosome 9 occurred in the long arm at about three-fifths of the distance from the end to the spindle fiber insertion region. Genetically the break shows about 14 percent of recombination with waxy and about 3 percent with v_1. The $wx-v_1$ interval therefore includes a region fairly close to the break. A few data were gathered on the effect of T 8–9 in herterozygous condition on recombination in this $wx-v_1$ interval. Based on 506 plants (grown only in the seedling stage), the recombination value is 18 percent. The data collected by CREIGHTON based on 1253 plants, given in table 3, show 14 percent. BEADLE (1932) found 8.3 percent for this same interval in a normal stock. Comparison with strictly comparable control material is necessary to determine if the observed increases are significant. The data are at least sufficient to show that T 8–9 causes no great decrease in crossing over in the $wx-v_1$ interval which includes a region close to the break. This failure to show any reduction is correlated with the observation in a later part of this paper that the interchange cross at prophase is very constant in its position, that is, that there is very little association of non-homologous parts. This correlation is to be expected if abnormal association is one of the causes of reduced crossing over in certain interchanges in regions adjacent to the break. Possibly T 8–9 will cause reduction in a very short region adjacent to the break. The tests are being continued.

TABLE 3

Four-point backcross data involving T 8–9 and factors in chromosome 9, furnished by Dr. H. B. Creighton.

GENOTYPE OF HETEROZYGOUS PARENT	PARENTAL COMBINATIONS	RECOMBINATIONS							
		REGION* 1	REGION* 2	REGION* 3	REGIONS 1 AND 2	REGIONS 1 AND 3	REGIONS 2 AND 3	REGIONS 1, 2 AND 3	TOTAL
$+wx+$ T 8–9 / $sh+au_1+$	407† 338	146† 139	83 60	34 38	11 7	7 2	7 4	3 1	
Total	745	285	143	72	18	9	11	4	1287
		24.6%	13.7%	7.5%	1.4%	0.7%	0.9%	0.3%	
$+wx+$ T 8–9 / $c+v_1+$	414 336	139 150	80 82	15 15	4 8	3 2	3 0	8 0	
	750	289	162	30	12	5	3	0	1251
		24.5%	14.1%	3.0%	1.0%	0.4%	0.2%		

* The percentages given for each of these regions include the proper double and triple crossovers.

† These figures are arranged systematically as follows: In each column the first number is of the genotype whose first gene is the one at the extreme upper left end. Thus, in the au_1 data for Region 1 there were 146 $++au_1+$ and 139 $sh\ wx+$ T 8–9.

CROSSING OVER BETWEEN THE MAIZE CHROMOSOME 9 AND ITS TEOSINTE HOMOLOGUE

In hybrids between Florida teosinte and maize, EMERSON and BEADLE (1932) found little or no recombination in the $C-wx$ interval. Since T 8–9 is at some distance from *wx*, it was crossed to a stock furnished by BEADLE carrying the $c-Wx$ chromosome of the Florida teosinte. This stock was the fifth or sixth generation resulting from backcrosses to *wx* maize in which the teosinte *Wx* chromosome had been saved in each generation. The results given in table 4 indicate that the amount of crossing over is not the same in different plants. Based on the ratios of *C wx* to *c Wx* (corn:teosinte), the plants appear to fall into two groups; one in which the ratio through the pollen shows only a small deficiency in the *c Wx* or teosinte class (42.8 percent of *c Wx*) and the other in which this class is extremely deficient (only 21.6 percent in the total from three such plants). In both groups, using the same heterozygous plants as the female parents, the ratios are approximately 1:1. In each case the crosses are exact reciprocals between the same two plants. In the group giving approximately normal ratios in reciprocal crosses, the recombination value with T 8–9 is 16.3 and 12.2 percent from the crosses using the heterozygote as the female and as the male respectively. This is to be compared with 4.3 and 2.3 percent in the crosses through female and male respectively in the group giving deficient ratios through the pollen. The difference between the two groups is significant. The differences in crossing over through female and male are not significant.

Backcross data from this cross of T 8–9 with Florida teosinte have been reported by BEADLE (1932). The F_1 plants used in his experiments were sibs of the ones used in mine. He reported a recombination value of 12.3 percent which agrees fairly well with my results from the group giving more nearly normal ratios through the pollen. The explanation of the low values in the other group is not known. Germination was low in both groups but was lower in the group giving the more nearly normal ratios of *C wx*:*c Wx* through the pollen. It is possible that the entire original teosinte *Wx* chromosome crossed over with its maize homologue only rarely. Since none of the long arm of chromosome 9 was marked genetically, part of this arm may have been replaced by a maize segment in the backcrosses by which the stock used here was established. Such crossovers might have occurred at different points in that arm, resulting in substitution of different amounts of the maize chromosome. Since two types appeared in this one cross, one such change would have had to occur in the immediate parent of the cross with T 8–9. In addition, to explain the low ratios through the pollen, a factor slowing up pollen-tube growth must be assumed to have been located in the part of the teosinte 9 chromosome be-

tween waxy (*wx*) and T 8–9. The loss of this section should result in a stock giving normal ratios through the pollen, and the recombination percent between T 8–9 and *wx* should be nearer normal. The tests need to be repeated in a cross of T 8–9 with pure Florida teosinte.

LINKAGE TESTS FOR CHROMOSOME 8

The determination of the linkage group carried by chromosome 8 which also would be expected to show linkage with T 8–9 was aided by McCLINTOCK's unpublished results on the association of several different trisomics with their linkage groups. Her tests finally showed that chromosome 8 carried either the sugary (*su*)—Tunicate (*Tu*) group or else it was the one for which no linkages had been found. (In the earlier note [BURNHAM 1930] aberrant ratios for red aleurone (*pr*) were reported from a trisomic plant carrying T 8–9 which suggested that this group might be involved. Direct tests showed that the *pr* group is not linked with T 8–9). Backcross tests of T 8–9 with *su* and with *Tu* showed independence, indicating that chromosome 8 must carry the missing linkage group. Japonica (*j*), an unplaced gene, was tested with T 8–9. This character was described as a Japanese strain and illustrated by a colored plate under the name *Zea japonica* by VAN HOUTTE in 1865–1867. The tests for linkage of *j* with T 8–9 (table 1) give conflicting data. When the semisterile plants were used as female parents, the progeny show linkage of *j* with T 8–9; when used as pollen parents they do not. This difference may not be very significant, since the crosses are not between the same stocks. A similar result has been reported, however, by ANDERSON (1934) and by RHOADES (1933). The combined data show 38.4 percent of recombination between *j* and T 8–9. Trisomic tests with *j* were made to determine whether it is in chromosome 9 or in chromosome 8. The test with chromosome 9 (2n+1, simplex for *j*, selfed) gave 209 *J*:73 *j*, a close fit to the 3:1 (70.5 *j* expected) from disomic inheritance. The trisomic test with chromosome 8 [+*j*(2n)×++*j* (2*n*+1)] gave 137 *J*:23 *j* in the *R* aleurone class and 83 *J*:13 *j* in the *r* aleurone class. In these cultures, the plants were tillered and the classification for japonica was clearcut in both aleurone classes. These numbers are close to the 5:1 ratio expected from trisomic inheritance and indicate that *j* is in chromosome 8. McCLINTOCK (1933) also has shown cytologically by means of deficiencies that *j* is in chromosome 8 and that its locus is toward the end of the long arm. Japonica must therefore lie in the piece of chromosome 8 which was translocated to chromosome 9.

Tests for linkage of T 8–9 with chocolate (*Ch*) which ANDERSON and EMERSON (1931) suggested might be located in chromosome 8 gave negative results, there being 222 parental:223 new combinations. The tests of *Ch* with *j* gave 225 parental:227 new combinations. In addition, tri-

somic tests with chromosome 8 (normal $ch \times 2n+1$ simplex for Ch) gave 77 chocolate:72 normal; a 1:1 or disomic ratio where 1:2 was expected

TABLE 4

Data from backcrosses to cwx of plants heterozygous for the teosinte cWx chromosome (9) and for the maize Cwx T 8–9 chromosome.

PLANT NUMBER	EAR RATIO			CLASS. FOR POLLEN STERILITY				
	C wx (CORN)	*c Wx* (TEOS)	PERCENT *Wx*	*C wx*		*c Wx*		PERCENT RECOMBINATION BETWEEN *wx* AND T 8–9
				SEMI-STERILE	NORMAL	SEMI-STERILE	NORMAL	
HETEROZYGOUS PLANT USED AS ♀								
2434–9	92	90	49.5	33	—	—	16	0
2465–5	25	24	49.0	16	—	2	8	7.7
608–10*	77	111	59.0	61	2	5	69	5.1
subtotal	194	225	53.7	110	2	7	93	4.2
2465–6	58	72	55.4	22	6	2	19	16.3
2464–4*								
subtotal								
HETEROZYGOUS PLANT USED AS ♂								
2434–9	133	56	29.6	52	1	2	17	4.2
2465–5	144	20	12.2	27	—	1	2	3.3
608–10*	141	39	21.7	89	1	—	19	0.9
subtotal	418	115	21.6	168	2	3	38	2.4
2465–6	111	83	42.8	19	5	3	20	17.0
2464–4*	99	74	42.8	41	2	3	24	7.1
subtotal	210	157	42.8	60	7	6	44	11.1

* Only segregating for $C-c$.

from trisomic inheritance. Therefore chocolate is not in chromosome 8. Similar trisomic tests of chocolate with chromosome 7 indicate it is not in that chromosome. Also backcross tests for linkage of chocolate with brown midrib 2 (bm_2) (32 parental:36 new combinations) indicate independence with this end of linkage group 1 which previously had not been tested with chocolate.

LINKAGE TESTS WITH FACTORS IN OTHER CHROMOSOMES

Considerable data were accumulated in tests of this interchange with genes in other linkage groups. At the beginning, nothing was known about the association of the linkage groups with particular chromosomes. In all cases, the data are from backcrosses. It is sufficient here merely to enumerate the factors that were tested without finding linkage: *chromosome* 1: Pericarp (P), fine striped (f_1), brachytic (br), anther ear (an), and brown midrib (bm_2); *chromosome* 3: anthocyanin (A_1), tassel-seed (ts_4), and dwarf

(d_1); *chromosome* 4: sugary (*su*), and Tunicate (*Tu*); *chromosome* 5: virescent (v_2), red aleurone (*pr*), and brown midrib (bm_1); *chromosome* 6: yellow (*Y*); *chromosome* 7: ramosa (*ra*), glossy (gl_1), virescent (v_8); and *chromosome* 10: aleurone color (*R*). Chromosome 2 was later eliminated by the cytological observation that T 1–2, when crossed with T 8–9, gives two separate rings of 4 chromosomes each (BURNHAM 1930).

Tests showing independence also were obtained with the gene albescent (*al*) which is supposed to be in chromosome 6. Other tests of *al* with purple (*Pl*) and of *al* with T 1–6b in which the break is in the satellite proper show no linkage. The gene *al* appears not to be in chromosome 6 unless the satellite shows a special crossover relation with the remainder of the chromosome, across the nucleole attachment.

In addition, the unplaced genes brown midrib-3 (bm_3), golden (g_3), and tasselseed (Ts_3) were tested with T 8–9 without finding linkage.

CYTOLOGICAL STUDIES

For cytological study T 8–9 was crossed with a stock which is homozygous for a large deeply-staining terminal knob on the end of the short arm of chromosome 9. Counts were made on the relative frequencies of rings and "chains" at diakinesis. "Chains" or strings of four chromosomes are infrequent, out of 108 clear figures, there were 7 such configurations, the remainder being rings. BEADLE (1932) observed 3 per cent of chains in similar material. At this stage, with the presence of the terminal knob on chromosome 9, together with the small differences in lengths, it was possible to determine where lack of association had probably occurred to break the ring into a "chain." In five of the seven "chains" observed, the short interchanged chromosome 8 was at the open end; probably indicating lack of association between the normal 9 and the piece of 9 which was translocated to 8. In the two remaining "chains" the normal 8 was at the open end probably indicating lack of association between the long arm of the normal 8 and the piece of 8 which was translocated to 9. The data are not sufficient to indicate the relative frequencies of the two types of lack of association, but do show that both may occur. At mid-prophase, MCCLINTOCK (1930) has shown that the interchange complex gives a cross-shaped figure when heterozygous, and that unequal terminal pieces have been interchanged. Chromosome 8 has lost more than it received, making it now much shorter than the normal 8; even a little shorter than chromosome 10 which in normal stocks is the shortest of the haploid set. The cross-shaped figure at prophase indicates that the interchanged pieces became attached at the broken ends. In two interchanges which have been reported (BURNHAM 1932, 1934), considerable variability in the position of the center of the cross has been found. Close association of the chromo-

somes was found in all the positions. Since the original breaks must have occurred at only one point in each of the two chromosomes, in every position except the one in which the center of the cross is at these points there must be association of non-homologous parts. In plants heterozygous for T 8–9, the position of the cross is very constant. Occasional figures show the cross at a markedly different position as is shown by the measurements of mid-prophase figures given in table 5. One of the 8 figures is of that

TABLE 5

Data from measurements of camera lucida drawings of mid-prophase meiotic configurations in plants heterozygous for T 8–9 (measurements in mm—magnification: ×4200).

SHORT ARM	LONG ARM			RATIO OF ARMS (LONG/SHORT)
	INSERTION TO "CROSS"	"CROSS" TO END OF ARM	PERCENT INTERCHANGED	
CHROMOSOME 8				
52	43	152	77.9	3.8
53	73	90	55.2	3.1
93	42	178	80.9	2.4
44	23	151	86.8	4.0
92	34	192	85.0	2.5
—	13	79	85.9	—
72	42	179	81.0	3.1
—	—	—	—	—
Average, excluding the second figure.			82.9	3.2
CHROMOSOME 9				
73	78	89	53.3	2.3
53	115	48	29.4	3.1
99	72	125	63.5	2.0
—	39	65	62.5	—
75	69	104	60.1	2.3
—	27	39	59.1	—
—	—	—	—.	—
115	64	128	66.7	1.7
Average, excluding the second figure			60.9	2.1

type. The average for the other seven gives about 83 percent of the long arm of chromosome 8 and 60 percent of the long arm of chromosome 9 as the pieces which were interchanged (this description should be less confusing than the use of the terms "right" and "left" end). These values are practically the same as those diagrammed by McCLINTOCK (1930) for this interchange. The corresponding values for the off-type figure are 55 and 29 percent respectively; that is, when the center of the cross is out nearer the end of the long arm of chromosome 9, it is also nearer the end of the

long arm of chromosome 8 by a similar amount. Such a result could not have arisen by mere stretching. The data in table 5 include only those figures in which the entire chromosome could be followed with certainty and do not indicate relative frequencies. Many figures were found and studied, from which it was concluded that the center of the cross is very constant in its position. In two interchanges, T 5–9 and T 2–6, which were studied cytologically and genetically the position of the center of the interchange cross at prophase was extremely variable (BURNHAM 1932, 1934). In each case this was associated with greatly reduced crossing over (BURNHAM 1934). In the T 8–9 reported here where there was very little variability, the $wx-v_1$ interval shows no reduction in crossing over. If the association of non-homologous parts, brought about by variability in the position of the center of the cross, prevents crossing over in these regions, the above correlations are expected, that is, great variability with greatly reduced crossing over and little variability with no reduction or reduction in a very short interval.

In the literature, DOBZHANSKY (1931) has emphasized competition in synapsis as a cause for the decreased crossing over observed in regions adjacent to translocations. No plausible mechanism for this competition has been offered. The variability in the position of the center of the cross suggests several possible mechanisms. MCCLINTOCK (1933) has reported evidence in maize that at meiosis synapsis has a tendency to begin at the ends of the chromosomes. There is no evidence to indicate that this process is initiated in different chromosomes at the same time, nor that it proceeds in all at the same rate. Where the chromosomes are in normal pairs, differences in these processes would still give normal synapsis. Where an interchange is present, a difference in either or both of these would affect the position of the center of the cross. Such processes might be expected to be variable, and would result in variable positions of the cross.

DEGREE OF STERILITY

Counts to determine the amount of pollen abortion were made by thoroughly teasing out about one-third of an anther in a drop of iodine-KI solution and counting the grains on the entire slide. The normal and aborted classes of grains are distinct, the aborted ones being practically devoid of starch, and somewhat smaller. Counts on twenty-five plants heterozygous for T 8–9 gave 25,135 pollen grains, of which on the average 59 percent were aborted. There was considerable variation, the standard deviation being 5.8 percent. The amount reported in the earlier paper (BURNHAM 1930) was 57 percent. Counts have not been made, but the ears show a comparable proportion of aborted ovules. In two or three plants out of the total number which have been examined for sterility during the

course of the work there were no visibly aborted grains, but 55 to 60 percent were small and well-filled with starch. Progeny were not grown from these to determine if they were heterozygous for T 8–9.

One culture was found to have plants with 67–69 percent aborted pollen in addition to plants with the usual 57–60 percent. McCLINTOCK (1933) has found an inversion in the short arm of chromosome 8 in certain of the maize stocks. She has observed irregularities in meiosis, and pollen sterility resulting from crossing over in the inverted region. Plants from the culture giving the higher sterility were examined cytologically at mid-prophase, diakinesis, and anaphase of meiosis with special reference to chromosomes 8 and 9. There was no evidence that an inversion was present. There may have been one in another chromosome or some factor affected distribution in the ring. This suggests the possibility that the plus deviation from 50 percent sterility observed in most plants heterozygous for T 8–9 may be due to the presence of an inversion. Several different cultures have been studied cytologically at prophase, none of which has shown an inversion. Nor have any cultures been found which show only 50 percent sterility.

The amount of pollen abortion (59 percent) is significantly in excess of 50 percent. McCLINTOCK (1930) found from cytological observations at the first microspore division in this interchange that any two chromosomes of the ring of 4 may pass together to one pole at anaphase I of meiosis; that is, that six classes of spores are formed. She has suggested that in half the cases, alternate distribution occurs, while the other half includes the two types of adjacent ones. Absolutely random 2 by 2 distribution in the ring of four should give 50 percent of spore abortion; since for each of the two "open" orientations, there should be a corresponding zigzag arrangement occurring with equal frequency. The former give aborted spores while the latter give normal ones.

An attempt was made in this material to determine the relative frequencies of the two types of non-disjunction, that is, 1, non-disjunction of the interchanged pieces and 2, non-disjunction of the non-interchanged pieces. Since only the alternate distributions, that is, zigzag arrangements, give normal spores, it is necessary to study only the "open" rings at metaphase. These are much easier to observe than the twisted or zigzag ones, but after considerable study it was concluded that the differences in chromosome lengths were not sufficient to permit identification in all cases of the two types of "open" ring orientation which lead to the two types of non-disjunction. Since the observed amount of abortion is 59 percent in place of 50 percent, it seems probable that the distribution is not a random one.

In Drosophila interchanges involving small pieces, DOBZHANSKY (1933) found that the type of distribution which resulted in non-disjunction of the greater amount of chromatin was less frequent. He also found that

when the breaks were nearly median, distribution of the four chromosomes was approximately random, showing that the spindle fiber is not the all-important factor determining chromosome disjunction. It may be that the spindle fiber is a region at which the forces first begin to act.

TRISOMIC INDIVIDUALS DERIVED FROM PLANTS HETEROZYGOUS FOR T 8–9

In the preliminary paper (BURNHAM 1930), results were reported from two 21-chromosome, low sterile plants which appeared in the progeny of plants heterozygous for T 8–9. Both gave ratios for factors in chromosome 9 approaching those from trisomic inheritance. One apparently was trisomic for chromosome 8 (27 percent sterile) and the other for 9 (33 percent sterile). The apparent trisomic ratios obtained from the former were due to the fact that *sh* on chromosome 9 shows about 33 percent of recombination with the interchange point. Later generations from the plant trisomic for chromosome 9 and heterozygous for T 8–9 have been studied. The probable genetic make-up of the three chromosomes is: (a) *C Sh wx* T 8–9, (b) *C Sh Wx*+, (c) *c sh wx*+. This plant was crossed to a *c sh wx*+ ♂, and the resulting low sterile (2n+1) plants were used both as the ♂ and the ♀ parents in crosses with *c sh wx*+. The low sterile, 21-chromosome plants may include four types: 1, a type which carries both interchanged chromosomes (T 8–9) and has two normal number 8 chromosomes and one normal 9 (that is, trisomic for chromosome 8); 2, one which has both interchanged chromosomes but has two normal number 9 chromosomes and one normal 8 (that is, trisomic for chromosome 9); 3, one which has a normal 2n complement but in addition has one of the interchanged chromosomes (that is, trisomic for a part of 8 and a part of 9) and 4, one which has a normal complement but in addition has the other interchanged chromosome. These last two types correspond to BLAKESLEE's "tertiary" types in Datura. For each interchange, it should thus be possible to isolate two such "tertiary" types. These may be obtained in subsequent generations from the cross of the interchange with the corresponding normal or "primary" trisomic stocks, or from 3–1 disjunction in a plant which carries the interchange. When either trisomic type 1 or 2 which carries both interchanged chromosomes is used as the pollen parent, the progeny should consist of normals and "semisterile" plants (heterozygous for T 8–9) with possibly an occasional low sterile as a result of the functioning of n+1 pollen. The data from such crosses are given in table 6. Only 17.9 percent (16.4 when the data are corrected for differential germination of *Sh* and *sh* seeds) of the offspring came from pollen carrying T 8–9. If disjunction were at random in the trivalent, 1/3 of the spores should carry T 8–9. This indicates that the two normal members of the trivalent disjoin more often than they

TABLE 6

Data from crosses of normal ♀ × trisomics carrying interchanged chromosomes from T 8–9. In the first group, those carrying both; in the second, those carrying only one of the interchanged chromosomes.

CROSS	POLLEN STERILITY OF THE PARENT TRISOMIC PLANT		PROGENY THROUGH POLLEN			
	NUMBER OF GRAINS	PERCENT ABORTED	LOW STERILE	NORMAL	T 8–9 (SEMISTERILE)	PERCENT T 8–9
N×460-1*	1392	25.7	7	246	41	14.3
N×460–9	1608	26.5	—	131	35	21.1
N×460–20	3173	28.0	—	174	33	15.9
N×461–23*	1500	29.8	1	210	56	21.1
N×465–3*	1747	29.8	1	171	38	18.2
subtotal			9	932	203	17.9
N×460–5*	2169	8.9	1	125	—	—
N×461–4*	2693	10.8	2	355	1	—
subtotal			3	480	1	—

* These plants were checked by root tip counts and were found to have 21 chromosomes (2n+1).

should on the basis of chance, at least at the interchange locus in the chromosome. If they disjoined from each other in every case, there would be no semisteriles among the 2n offspring. If the three chromosomes are represented as: (a), (b), (c), of which (a) carries the translocation while (b) and (c) are normal, and if the proportions among the three types of synapsis are assumed to be 4 bc:1ab:1ac, about 16.7 percent of the 2n spores will carry T 8–9. This is very near to the observed percent, but disjunction is probably complicated by the fact that most of the figures are chains of 5 chromosomes. Pollen sterility counts were made on 87 of the "semisterile" plants obtained from the above crosses to determine if they were similar to the original plants heterozygous for T 8–9. Plotting their frequencies by 2 percent intervals shows a slightly bimodal distribution, one mode falling at 58 to 60 percent while the other falls at 62 to 64 percent. Although the difference is not based on large enough numbers, it suggests that there is a group showing higher pollen sterility. The presence of an inversion is one possible explanation for this group. The plants in the other group show a degree of pollen sterility similar to that in the original plants.

The two types of trisomics which carry one of the two interchanged chromosomes as the extra one should give entirely different results. Through the pollen, only normal progeny should appear except where an n+1 grain functions to give a "low sterile" plant. Two such 21-chromosome plants which showed very low pollen sterility (8 to 11 per cent), and which gave abnormal or trisomic ratios for shrunken (*sh*) were tested (last two lines in table 6). There were 480 normal offspring and only one "semi-

sterile." The latter probably was a stray pollination. It will be noted in table 6 that a few "low sterile" plants also appeared, about 0.74 percent. Pollen counts made on these plants determined for certain that they were "low sterile." They undoubtedly arose from the functioning of n+1 pollen. No data have been obtained on the frequency of n+1 through the pollen of normal trisomics of either 8 or 9 for comparison.

Abnormal ratios for factors in chromosome 9 were obtained from many of the trisomic plants. One plant which showed 11 percent of pollen abortion gave only 11.3 percent of *Sh* seeds through the pollen in a backcross to *sh*. By assuming the correct amount of preferential pairing and lagging, such ratios can be explained, but until more is known about the actual distribution there is little value in such an attempt.

SUMMARY

1. The interchange between chromosomes 8 and 9 (T 8–9), referred to earlier as "semisterile-2," shows 13.7 percent of recombination with waxy (*wx*), 32.9 percent with shrunken (*sh*), and 34.2 percent with colored aleurone (*C*). CREIGHTON's data which are included show 7.5 percent with aurea$_1$ (*au*$_1$), and 3 percent with virescent$_1$ (*v*$_1$). The order is *C*−*sh*−*wx*−T 8–9 with *au*$_1$ and *v*$_1$ also probably between *wx* and T 8–9.

2. No reduction in crossing over was found in any of the regions studied. Part of the *wx*−*v*$_1$ interval is probably in the arm in which the break occurred.

3. The gene for japonica stripe (*j*) is in chromosome 8, and shows 38 percent of recombination with T 8–9. That for chocolate pericarp (*Ch*) is not in this chromosome.

4. Crossing over with the Florida teosinte chromosome homologous with the maize chromosome 9 was observed in the *wx*−T 8–9 interval as was reported by BEADLE, but the amount varied. This seems to be associated with some factor upsetting genetic ratios through the pollen.

5. Pollen counts show about 59 percent of aborted pollen in plants heterozygous for T 8–9. There is a comparable degree of ovule abortion in such plants. Plants homozygous for the interchange show normal fertility.

6. Counts at diakinesis in plants heterozygous for T 8–9 showed about 7 percent of chain configurations, the remainder being rings of four chromosomes.

7. Mid-prophase figures show that the position of the interchange "cross" is very constant. Only occasional figures show it in a widely different position. It is pointed out that in the two chromosomes involved in an interchange, a variation in time of initiation of synapsis or a variation in

its rate of progress along the chromosomes would bring about variability in the position of the "cross."

8. Genetic tests of a plant which was trisomic for chromosome 9 and which carried T 8–9 (a chain of five chromosomes) showed that synapsis must have been preferential between the two normal homologues of the group.

9. Tests through the pollen or trisomics carrying only one of the interchanged chromosomes gave mostly normal offspring and no "semisteriles." There was a small percentage of 2n+1 low sterile plants.

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THE MORPHOLOGY OF THE X CHROMOSOME IN SALIVARY GLANDS OF DROSOPHILA MELANOGASTER AND A NEW TYPE OF CHROMOSOME MAP FOR THIS ELEMENT

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In a paper which appeared recently in this journal the writer has described a new method for the study of chromosome structure and chromosome aberrations and has called attention to its application to a number of problems in the field of cytogenetics. In the present paper, the normal morphology of the X chromosome will be described and a new type of chromosome map will be presented showing the approximate position of some 21 gene loci scattered well along the whole active region of the X. It is believed that this type of analysis marks a distinct advance in the technique of cytogenetics and will be of general interest from both practical and theoretical standpoints. On the practical side, the new chromosome map of the X may be used as a yardstick to determine the genetic position of new breaks cytologically, thus greatly simplifying the breeding tests which need be made and saving much time. From a theoretical standpoint, perhaps the most significant point is the demonstration that we have in our hands a qualitative cytological method of chromosome analysis which should be applicable not only to Drosophila but other types of insects and perhaps to other animals as well. For if the X chromosome of *D. melanogaster* has such distinct topographical features that we can follow a section as it is translocated here or there through the agency of irradiation, there is no obvious reason why the same method of study may not be applied to a study of all the chromosomes not only in this species but in related forms, thus opening up to a direct cytological attack the whole field of the behavior of chromosomes in phylogeny and the hosts of questions clustered about this central theme. Of considerable interest, also, is the discovery that neither the inert region of the X, nor the Y chromosome, except possibly for a short piece, which probably carries the locus for bobbed, are visible as recognizable formed structures in salivary gland cells. The X appears to be made up wholly of the genetically active region.

MATERIALS AND METHODS

Many of the breaks considered in the following pages are new ones obtained by my colleagues Professor J. T. PATTERSON and Mr. WILSON STONE, and by Miss BEDICHEK, during the course of a series of rayings carried out in their attack on the position of a primary sex factor, and

have not been described before in genetic literature. I am indebted to my colleagues for the use of this material, and especially to Mr. WILSON STONE who has carried out many genetic tests to determine the position of new breaks, and who in various ingenious ways, has built up stocks needed by the writer during the course of this study.

The exact determination of the point at which a chromosome has broken depends, among other things, on the markers used in the original experiment. For this reason the known gene loci which bound a break may be a number of crossover units apart. Under these conditions the cytologist can do no more than say that the two limiting loci are to the left and right of a given morphological point. In some cases the position of the break is known within two-tenths of one crossover unit, thus enabling us to plot the morphological position of genes with relative exactness. Realizing this limitation of our method, the writer has assigned to one of his students, Mr. OTTO MACKENSEN, a study of short deficiences of known genetic character. As this is written the results of Mr. MACKENSEN indicate that this method will allow us to locate genes within very narrow limits, perhaps on a given band. When this is done we will be able to present a more refined and accurate map of the active region of the X.

In my first paper I gave the general procedure of preparing the salivary gland for study, but since some of my readers may want to confirm or extend the observations here recorded, I am giving below a somewhat fuller account of the method.

Old larvae, about ready to pupate, should be selected for study. In younger larvae, the union of homologous chromosomes is usually not as complete nor the nuclei as large, as a rule. Dissection is made in Ringer's solution (Frog formula) with a binocular dissecting microscope, and a black background under the dish aids in finding the salivary glands. Male larvae may be distinguished by the fact that at about two-thirds the length from the head, in dorsal view, the gonads may be seen through the body wall as symmetrically placed, large clear spaces. In females the gonads are very small and are not easily visible through the body wall. Larvae are torn open with needles at about their middles. This causes the viscera to extrude. The salivary glands are transparent organs attached to the pharynx near the jaws. They may be recognized, both by their transparency and by the fact that on one side of each there is usually a narrow border of fatty tissue while the opposite side is more or less attached to the ladder-like fat body. It is desirable to free the glands of the fat, though little time should be given this process. The glands are transferred to a clean slide with a pipette, then the Ringer's solution removed. Iron-aceto-carmine is now applied. If it is flowed on from one side the glands will usually stick to the slide making subsequent manipulation easier. Cover

the glands and stain with a thin cover glass (preferably .15 mm or less in thickness so that it fits the slide snugly) at once, and crush the salivary gland by pressing on top of the cover glass with a needle. This process will free many nuclei from their surrounding cytoplasm, thus making observations later much easier. It also usually squeezes most of the aceto-carmine from under the cover glass. It is wise, after crushing, to pry up on the cover glass a trifle so as to bathe the gland tissue freely in the stain. Be careful to exclude all air bubbles. Stain for from 10 to 20 minutes. The exact time is probably not so important, but if the day is hot and dry it is perhaps wise not to allow the aceto-carmine to evaporate too much. A little evaporation seems desirable. The excess stain is now removed with filter paper and then under the high power of the dissecting microscope the individual nuclei are crushed and the chromosomes more or less spread out, by pressing on the cover glass with a needle. Again blot the preparation with filter paper using plenty of pressure and seal the cover glass with vaseline. Such a preparation may be examined at once but it is better after a few hours. These preparations are not permanent, but they last well for a week or ten days, if air is excluded.

A few comments about the preparation of the stain and the examination of slides may be helpful to those who have not used aceto-carmine for this type of work. It has been the experience of the writer that not all carmine works equally well in the preparation of aceto-carmine nor am I always successful in preparing a good lot of stain. Carmine No. 40 is very satisfactory and has been used exclusively. In preparing the stain (simmer an excess of powdered carmine in a forty-five percent solution of acetic acid and, after cooling, filter) care is taken not to allow much loss of fluid during the heating. After the stain is cool and has been filtered, the writer places a dissecting needle or an old file in the bottle of stain so that a trace of iron will be dissolved by the acid. It will be noted that after a few minutes the aceto-carmine will have turned appreciably darker. The needle is then removed.

The examination of aceto-carmine preparations is greatly facilitated by the use of artificial light tinged blue-green. For this purpose one may insert, between the light source and the microscope, a ground-glass screen and a flask containing a dilute solution of copper sulphate (*without ammonia*), or, one may use a frosted blue-green glass filter. Neutral filters of 10 and 20 percent absorption capacity, are extremely useful in controlling the intensity of illumination.

MORPHOLOGY OF THE X CHROMOSOME

For a general description of the morphology of the chromosomes in salivary glands the reader is referred to a recent article, by the author, in

this journal. Here we need mention that, in the salivary glands of old larvae, homologous chromosomes undergo somatic synapsis. Hence, in describing the X, in females, we are really dealing with two X elements which are so closely joined throughout their lengths as to appear as one chromosome. In such cases we shall speak of the X's as if they were a single body.

Figure 1 shows the typical structure of the X chromosome.[1] This is really a composite drawing, each segment representing a camera lucida drawing taken from some preparation in which the region concerned showed clearly and without distortion. My purpose has been to place before the reader a chart from which he should be able to identify any region of the X, on the examination of a good moderately stained aceto-carmine preparation. In such preparations the thickness of the X chromosomes varies a good deal depending on the state of contraction, excellence of preservation and doubtless other factors, but in general the longer the chromosome the thinner it appears and *vice versa*. In figure 1, I have sought to retain representative proportions but in putting together segments of different drawings no doubt errors have been made both in the length and in thickness. Such errors should be both plus and minus, and thus largely cancel each other. In any event they do not affect the qualitative character of the segments which is the point of primary interest. It may be said that the size of the double plate is due to the length of the right arm of the third chromosome, a map of which is in press in this journal. Thus a uniform magnification for all elements will be maintained in this series of studies.

Above figure 1 two crossover maps have been indicated. The one immediately above the figure is the new standard map which places the nor-

[1] Through the courtesy of the editor I have been allowed to substitute for the original figure 1 a new topographical map of the X, which incorporates many of the finer details of morphology which have come to light with various improvements in technique, and gives additional evidence for the morphological limits of gene loci. The present figure supercedes the earlier one published in SCIENCE (1933) from which it differs in some details and represents a map of the X as it stands at the end of March, 1934. Two aberrations, not discussed nor figured in the text, are plotted (scute-8 and X–IV, III. 12). Recent work at this laboratory (PATTERSON and STONE, in press) has shown that scute-8 is broken between the gene loci for achaete and scute. Morphologically, the point of breakage is shown at the left in figure 1. In the stock known as X–IV. III, 12, obtained by STONE, there has been an extensive rearrangement of the X chromosome. Scute and echinus are separated by one break, the gene for cut has been deleted and another break is between the genes for garnet and scalloped. These enable us to bracket the gene loci for scute, cut and garnet more closely than before. Two corrections are incorporated in figure 1. The left hand limit of the ClB inversion is not exactly the same as delta 49, but is a little farther to the left as shown. Also the point of breakage of the X–IV III, 9 is three bands farther to the right than shown in SCIENCE.

Two crossover maps are given. The New Standard Map values representing the X as 75.5 units long is next to the X. Above I have plotted the crossover values as given by BRIDGES and DEMEREC in their "Drosophila Information Service" Number 1, March 1934.

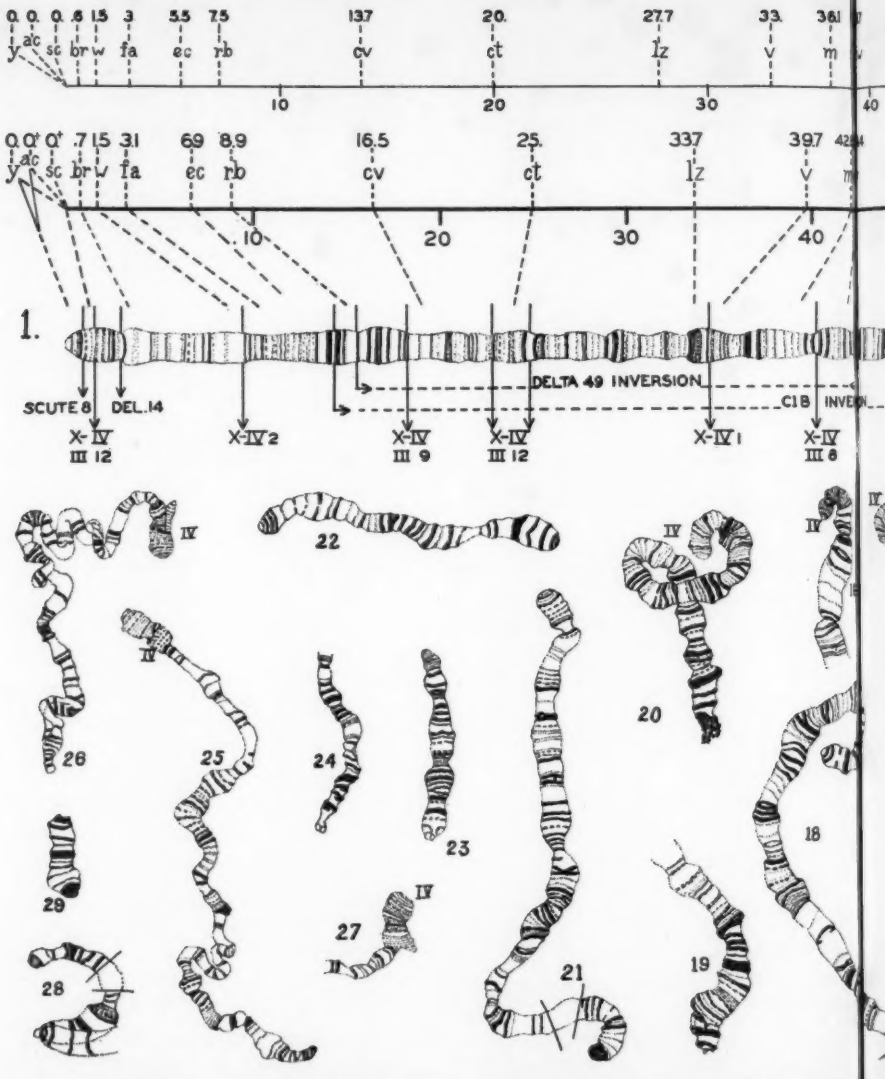
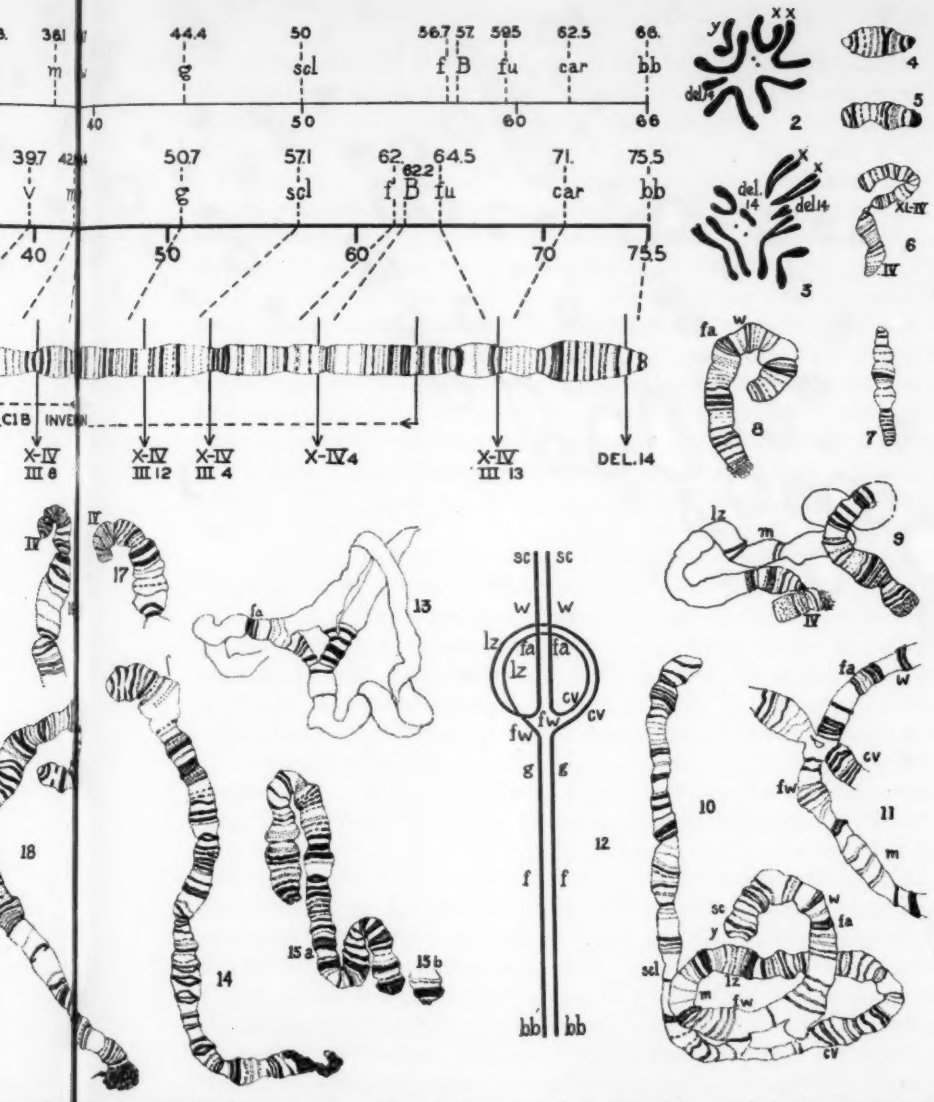


FIGURE 1.—Typical morphology of the X chromosome, as seen in the salivary glands of female larvae (see note) morphological positions have been placed by this study. A line running from the symbol towards the X sh the aberration, is indicated just below this drawing. FIGURES 2 and 3.—Oogonial metaphase plates to show the s of de from a female which carried two deleted X's and a Y chromosome. FIGURES 4 and 5.—Deletion 14 as it appe in s location. FIGURE 8.—The XL portion of the X produced by the X–IV, III. 9 break. FIGURE 9.—The IV- sect X chromosome. FIGURE 11.—The character of the pattern at the point of divergence between normal a delt FIGURE 13.—Shows the way a normal and a ClB X chromosome synapse, with most details omitted. FIGUR 4 to the XR–IV component, in whole or in part. FIGURES 20 and 21.—The XR–IV and the XL–IV translocation ob ed FIGURES 23 to 26.—The forked-Bar break. Figures 23 (female) and 24 (male) show the XR, and figures 25 and the chromosome. FIGURE 29.—The XR element of the X–IV, III. 13 break.



arvae (see note on p. 451). On the crossover maps above the X, we have the symbols of the gene loci whose approximate s the X sh the general area of its locus. The morphological position of the break in the X, together with the names of the show the sf deletion 14 as compared to the X and the other chromosomes. Figure 2 is from an attached X female, figure 3 4 as it appe in salivary gland cells. FIGURES 6 and 7.—The XL-IV chromosome which resulted from the X-IV. 2 trans- —The IV- section. FIGURE 10.—The inversion figure obtained from a female heterozygous for a normal and a delta 49 normal a delta 49 X's. FIGURE 12.—Schematic representation of the union of a normal and a delta 49 X chromosome. d. FIGUR to 19.—Various details of the X-IV 1 translocation. Figures 14 and 15a represent the XL piece, figures 16 to 19 location o ed from the X-IV, III. 8 break. FIGURE 22.—The XR element obtained from the X-IV, III. 4 translocation. ures 25 an the XL-IV elements. FIGURE 28.—The XR segment of the forked-Bar break with some indication of a Y

mal allele of bobbed at 75.5. This map will be used in the following description. At the top of the plate the latest crossover values for the X are indicated as these are given in the Drosophila Information Service (DIS) No. 1. The labels immediately below the X indicate the name of the breaks considered in this study.

The X is segmental in character and it greatly facilitates descriptions to give these segments names, even before presenting the evidence to show why they are so named. The constrictions which limit segments vary in depth and are subject to some variation; some are very constant.

The left hand end of the X terminates in a highly characteristic oblong segment which shows nine lines or bands clearly, none of which is, relatively speaking, either broad or deeply staining. Next to this end segment is an area relatively free from conspicuous bands. To the left we have an enlargement of the matrix forming a bulge or vesicle. The latter is very constant in position but varies a good deal both in size and appearance. Typically, we find dash-like lines running across or around the middle, but at other times it appears only somewhat clouded or even quite clear. A narrow collar separates this vesicle from the yellow-scute segment.

The next landmarks along the X are two areas, the first consisting of two bands which, as I shall show, are near the right hand limit of the locus for white. The second is composed of five narrow bands, the last of which is dash-like, which we shall call "facet" bands because Mr. MACKENSEN (1934) has found that when facet is deleted certain of these bands are absent. These landmarks vary somewhat in appearance. In chromosomes relatively contracted and deeply stained they appear as rather broad but not sharply defined or deeply stained areas. When the X is elongated we can see the thin individual lines more clearly and the banded effect is less conspicuous.

A little distance to the right of the facet area are three prominent bands the first of which is rather narrow and deeply staining, the other two distinctly double in their make up. Next come a set of six or seven prominent bands. The first, which lies near the left hand limit of the delta 49 inversion may stain deeply, when it is conspicuous, or, diffusely, when it it not prominent, but the next six are very constant in appearance. The first appears single, but stains deeply with the sharply defined edges, the second is double and appears relatively very broad, the third is like the first, and then there are three narrow bands lying close together which bound this segment at the right. Following the "six-banded" area there are some six segments all more or less banded but with no outstanding topographical features. This region is bounded at the right by what will be called tentatively the "lozenge" segment. Here I wish to point out that the appearance of this segment may vary in different larvae. Usually, a

number of individually narrow lines are crowded together at the left end of this segment forming a very broad banded area, but sometimes, in what are presumably normal X chromosomes, the lines at this end are pushed to the right and the banded effect is lost.

At the right of the "lozenge" segment we have, perhaps, the most easily identified section of the whole X. At the left of the segment are two broad and heavily staining bands, the first about twice as thick as the second. At the right end are two much narrower but sharply staining bands. In between these boundaries the lines are thin and faintly staining. This segment has been named the "miniature" segment and its right end seems to be structurally a weak spot in the X, for there is a very pronounced tendency, with the technique employed, for the chromosome to break here.

The segment just to the right of the miniature area varies in different preparations. Typically, it is as shown but in some slides there is a marked tendency for the lines to crowd towards the left end forming a conspicuous band.

The next conspicuous area lies just to the right of the X-IV-III.4 break. There is a segment here bounded at the left by four narrow but sharply staining lines. Just to the right of this four banded area the next segment usually shows two conspicuous dash-like bands. This is called the "forked" segment because a break between forked and Bar breaks the chromosome at the right end of this segment.

The whole right end of the X chromosome beyond the forked Bar area has a highly characteristic and conspicuous topography. First, we have five deeply staining and broad banded areas with a definite number of lines forming each band. To the right of this area we have a clitellar-like enlargement of the matrix, then a narrower neck region followed by a greatly enlarged right-hand end terminal segment. This end segment is much subject to distortion and, hence, variation in appearance. One of the commonest distortions is for the segment to tear apart in the region just to the left of the three last bands. In males the fainter lines of this segment usually do not show distinctly. The paired X's terminate to the right either in two ring-like stumps, or in two fine protoplasmic strands which connect them with the chromatic coagulum or chromocenter.

The foregoing description of the morphology of the X is based on a study of female larvae in which the X's are paired and as thick in diameter and as deeply staining as any of the autosomes. In males the X shows the same regional differentiations as in the female but the chromosome is much thinner, stains much less deeply and is more apt to be contorted by the technique. It should be remembered that in the female the X is paired while in the male the X is single, the Y not being visible

with the technique employed. Many of the drawings given below are from male larvae, which accounts for the thinness of the chromosomes as compared to the paired element shown in figure 1.

Deletion 14

This aberration has proved to be the most illuminating of all those studied and will be considered in some detail. It has already been described both cytologically and genetically (PAINTER and MULLER 1929, MULLER and PAINTER 1932). It is a case in which nearly the whole of the genetic map has been deleted leaving the two ends of the X joined together. The break at the left end is between scute (0.1) and broad (0.7) and at the right hand end between carnation (71) and the normal allele of bobbed (75.5), the latter being carried by the deletion according to the genetic analysis of Dr. MULLER. In figures 2 and 3 oogonial plates are shown from which the size of the deletion, as compared with normal X's, may be seen. An explanation of the figures is given in the legend. The deletion has a volume of between a third and a fourth of that of the normal X, its large size being due to the fact that it carries a large amount of relatively inert material in its right hand end, and it is this inert material which makes it appear so large in oogonial plates (PAINTER 1931b, MULLER and PAINTER 1932). A study of deletion 14, therefore, should show us a number of interesting points: first, the contribution made from the left hand end of the X, thus giving us morphological limits for the loci of scute and broad, second, the general position of the normal allele for bobbed, and third, that region of the X which is genetically inert, since a considerable portion of this is represented in metaphase plates.

Figures 4 and 5 represent deletion 14 as it appears in salivary gland cells. By comparing these figures with the left hand terminal segment of the X of figure 1 the reader will have no difficulty in identifying that portion of the deletion which comes from the left end. The whole terminal bulb is represented with the exception of the collar which separates this bulb from the vesicular area to the right. The two dark bands of the nipple-like projection, in figure 4, are interpreted as the two terminal bands from the right hand end of the X, as may be seen by an examination of figure 1. From this evidence, it is clear that scute lies in or on the left terminal bulb, and that broad must be to the right of the break, as shown in figure 1. Likewise, the normal allele for bobbed must lie on or about the two terminal bands at the extreme right-hand end of the X. The inert region either is not present or it is represented by the nipple-like right tip of deletion 14. In passing, it may be noted that the volume of deletion 14, as seen in salivary glands, is about 1/20th of the X, while in metaphase plates it is fully 1/4, or more, of this element. The bearing of these facts

will be discussed later in this paper, in connection with other data.

Translocation X-IV 2

This stock, commonly known as 'mottled-5' at this laboratory was isolated by MULLER and analyzed genetically by BOLEN (1931). A mutual translocation between the X and a fourth chromosome is involved. The X is broken between the loci of white (1.5) and facet (3.1). In salivary gland nuclei, the break in the X is to the right of the first two fairly prominent bands in the sub-terminal segment (figures 6 and 7). In figure 6, which shows the XL-IV element in close association with a normal fourth, these bands can be seen, but in figure 7 the emphasis of the lines is not so clear. In figure 1, the approximate location of this break is shown. It is possible that the locus for white may lie close to or on the first of the "facet" bands because MACKENSEN (1934) has found that a short deletion removing this line also removes the locus of white.

Translocation X-IV III-9

This is one of the translocations recently isolated by my colleagues, PATTERSON, STONE and Miss BEDICHEK, and incidentally was analyzed cytologically before a genetic analysis was made. From cytological data, I came to the conclusion that the break was close to crossveinless, and genetic data since obtained by STONE show that it is between the gene loci of echinus (6.9) and crossveinless (16.5). Cytologically, this proves to be a mutual translocation in which most of the fourth chromosome is attached to the left end of the XR component. Figures 8 and 9 were taken from homozygous females. Figure 8 shows the XL-IV piece and we note the presence of the three heavy and two light lines of the six-banded area. Figure 9 shows the IV-XR piece and by following the pattern in the region of the fourth chromosomes we can easily identify the peculiar bands which lie under 20 of the crossover scale on figure 1, with several additional bands to the left. From these figures it is clear that the original X was probably broken between or very close to the fifth and sixth bands of the six banded area, as is shown in figure 1. This places echinus to the left of the break, while crossveinless must lie somewhwere to the right of it. Here it may be pointed out that the crossover value of crossveinless is close to its morphological left limit. That is, morphologically the left hand limit of crossveinless is about 18 units from the left end while its crossover value is 16.5. In contrast to this, we note that the other gene loci at the left end of the X have morphological values in linear units far in excess of their crossover values. Thus the left limit of facet is some ten linear units from the left end morphologically, while its crossover value is 3.1. From crossveinless on to the right hand end of the X, there is a close agreement of crossover and cytological values.

The delta 49 inversion

This inversion has been analyzed genetically by MULLER and STONE (1931), with some additional data presented by GLASS (1933). The left hand limit of the inverted area is between ruby (8.9) and carmine (22.5), and the right hand limit was first reported as being between furrowed (43.4) and forked (62). Subsequently, it was determined (unpublished data) that the right hand break was between furrowed and garnet (50.7). In spite of these rather wide genetic limits, the rearrangement is of value when considered along with the other observation described in this paper.

Female larvae heterozygous for a normal and a delta 49 X were studied. Figure 10 shows the way these two X chromosomes are joined. Figure 11 is given to show the morphology of the inverted area, and figure 12 is a simple diagrammatic interpretation of this case. The left hand limit of the inversion (figures 10 or 11) is in the clear or achromatic space between the three-banded and the six-banded areas. In figure 11 we see that the two X's synapsed both at the three-banded and the six-banded areas while between the elements diverge. Figure 10 shows the same thing somewhat less clearly because some of the lines are stretched and thus appear less deeply stained. From figure 1 we see that the point at which the delta 49 chromosome is inverted is to the left of the III-9 break already described, hence it is clear that genetically the break is to the left of crossveinless. From genetic data we know that ruby (8.9) is outside of the inversion, so that by combining genetic and cytological data we can say that the delta 49 chromosome is inverted very close to 12 crossover units from the left end.

The right hand limit of this inversion, morphologically, is at or very near the end of the segment which lies immediately to the right of the miniature segment (figure 10) and which is shown diagrammatically in figure 1. Since furrowed lies within the inverted area, we have the right-hand limit for this locus, and since garnet lies outside of the inversion, we know that its locus is to the right of the break.

The ClB inversion

A partial genetic analysis has been made of this gene rearrangement by MULLER and STONE (1931). They concluded from a study of a partially reinverted chromosome, that the left limit of this inversion was somewhere to the left of cut (25). Nothing very definite is known about the genetic right hand limit of this inversion, although some unpublished data recently obtained by STONE suggests that carnation lies outside of the inverted area.

Females heterozygous for the ClB chromosome were studied cytologically. The left limit of the inverted area is very close to or between the two

heavy bands of the three-banded area thus lying a little to the left of the point where delta 49 was inverted as is shown by figure 1. Figure 13 shows the points of the inverted areas. From the evidence presented for the delta 49 chromosome we are able to say that the left limit of the ClB inversion is very close to 12 crossover units from the left end.

The right-hand limit of the ClB inversion is near the right-hand end, cytologically, and comes between the second and the fourth of the five heavily banded areas, as shown by figure 13, or diagrammatically in figure 1. From evidence to be presented, this point is genetically between Bar and carnation.

Parenthetically, it may be pointed out that the union of the two elements in a larvae heterozygous for a normal and a ClB chromosome, may be very complete. This raises the question, is the union in meiosis as intimate, and if so, why does not more crossing over occur in the very long inverted section? If we assume that the meiotic pairing is like that we see in the salivary gland, then the following observations may throw some light on these questions. In any given preparation of a salivary gland, the union of homologues will be seen in many different stages, from cases with complete union, except at the points of inversion, to young cells in which only the spindle fiber ends are joined. It is not unusual to find cells in which both right and left ends of the X chromosomes are joined for some distance before the middle area is synapsed at all. With the two ends anchored in this way, there would be little opportunity for the strands of homologues in the inverted area to twist about each other. In so far as crossing over is dependent upon a twisting of the strands, this would seem to be a factor in reducing the amount of crossing over.

Translocation X-IV 1

The case was found by MULLER in 1928 and was analyzed by him genetically in collaboration with STONE. From the genetic evidence, it was supposed that a long section from the middle of the X had been deleted, with the subsequent attachment of this deleted area to a fourth chromosome. The left-hand break was between lozenge (33.7) and vermilion (39.7) and the right hand between the normal allele for bobbed and the spindle fiber attachment. Thus, the gray or left-hand part of the X was supposed to have the spindle fiber of the original X from which this aberration arose, while the deleted area carrying Bar was supposed to have obtained its spindle connection from the fourth chromosome to which it became translocated. Cytological evidence would indicate that these inferences, drawn from genetic data, regarding the right-hand end are not well founded. This case will be considered in some detail.

The reader will doubtless find it easier to follow the description given

below if he will first study the morphology of the two segments of figure 1 which lie between the labels of *lz* and *m*. It will be noted that in the lozenge segment a number of rather narrow bands are crowded towards the left end forming a broad band, while the miniature segment is characterized by the presence of four heavily stained bands, a pair to each end.

Figures 14 to 19 were all taken from female larvae homozygous for the X-IX 1 translocation. Figures 14 and 15a show the morphology of the XL or gray component. By comparing these with figure 1 we see that all the typographical features of the left end of the X are represented up to and including the broad band of the lozenge segment. Here the gray or XL element ends in a spindle fiber attachment vesicle or granule, shown in figure 15b, with no trace of any bands such as we see at the extreme right hand end of the X chromosome (figure 1).

The Bar component (above in figures 16 and 17) carries all of the banded part of the fourth at its left-hand end. Following the fourth chromosome part, in figures 16 and 17, we have the remainder of the lozenge segment and then the striking pattern of the miniature segment. In figure 18 the fourth chromosome part is not shown but the rest of the XR piece is present and by comparing this with figure 1 we are able to recognize all features up to and including the bands which are adjacent to the point of spindle fiber attachment. The last feature is best seen in figure 19. As far as I can determine, the spindle fiber area of the XR chromosome carries all the bands it should but it generally is somewhat distorted so that in the end it may have suffered some changes.

This X-IV 1 translocation allows us to give morphological limits to the loci of the lozenge (33.7) and vermilion (39.7) as shown in figure 1, but it does not help us place the normal allele of bobbed. From the cytological evidence, this aberration does not appear to be a long deletion accompanied by a translocation, such as MULLER and STONE first thought (see page 337, MULLER and PAINTER 1932). Rather it looks as if this is a mutual translocation in which just the spindle fiber part of the fourth chromosome has become attached to the XL component, while the rest of the fourth chromosome is attached to the left end of the XR element. In this process some of the fourth chromosome, perhaps, has been lost. This case deserves more study both genetically and cytologically.

Translocation X-IV III-8

This aberration was isolated recently by my colleagues. The genetic analysis by STONE shows that a break occurred between the loci of miniature (42.8) and garnet (50.7). Both cytological and genetic evidence show it to be a mutual exchange. Figure 21 was taken from a female homozygous for this translocation. The XL piece is represented and by comparing this

with figure 1 we see that all the left hand portion of the X is present up to and including the four bands of the "miniature" segment. The fourth chromosome has made very little morphological contribution to this XL piece except the spindle fiber area. The XR piece, figure 20, shows almost the whole of a fourth chromosome attached to the left-hand end. In other respects this piece is normal in topography from the point of break to the spindle fiber. The point of this break is shown diagrammatically in figure 1, and gives us limits for the loci of miniature and garnet.

Translocation X-IV III-4

This is a new translocation recently obtained by my colleagues and the genetic analysis by STONE shows that the X is broken between scalloped (57.1) and forked (62). Cytologically, we find that the break occurred just to the left of the four-banded area. Figure 22 shows the X R component. By comparing this with figure 1 we see that from the point of breakage on to the spindle fiber, the XR piece is normal. This case gives us morphological limits for scalloped and forked.

Translocation X-IV 4

This most interesting case was found by Mr. STONE and analyzed by him. The X is broken between the loci of forked (62) and Bar (62.2), the forked or gray section being translocated to a fourth chromosome. Figure 23 is from a homozygous female, and figure 24 from a male, showing the XR section of the X chromosome. It will be seen that the X is broken just to the left of the segment which is characterized by the five broad-banded areas. The segment where the break occurred (figure 1) shows two dash-like bands. As far as I can tell, the break was to the right of these bands, but a little of the achromatic section is still visible, to this a fragment of a fourth chromosome is attached. The XL-IV chromosome is shown in figures 25 and 26, with an additional terminal area shown in figure 27. These figures were from male larvae, and while it is somewhat difficult to identify all portions of the X up to the point of breakage, we get an excellent idea of the relative lengths of the XR and XL components. In addition we can see very clearly just how much of the fourth chromosome is missing. This case allows us to place morphological limits to forked and Bar and is further illuminating for several other questions which will be taken up later.

Translocation X-IV III-13

This is another case found recently by my colleagues and analyzed by Mr. STONE. It is of unusual interest because it enables us to place carnation. The break occurred between the loci of fused (64.5) and carnation (71). The XR section is shown in figure 29. Here we note the right and

terminal segment and the segment to the left of it. There is a dark bump on the left end of this XR piece. I have been unable to determine whether this is a small part of the next adjacent segment of the X, which I have called the "clitellar" segment, or whether it is a small piece of the fourth chromosome. In figure 1 I have placed the break through the right end of the clitellar segment. Fused lies to the left of this break and carnation must lie to the right. It is interesting to note that the crossover value of carnation is 71 and its position in morphological units can not be farther to the left of the spindle fiber than six and a half, or perhaps it would be clearer to say it lies at least 69 units from the left end.

DISCUSSION

This study of the X chromosome confirms the two main conclusions presented in the first of this series of papers, namely, (a) that in salivary glands of old larvae homologous chromosomes undergo somatic synapsis during which the union of the two elements is so intimate that one apparent structure is formed, and (b) that each chromosome has a definite and characteristic morphology which enables one to identify the same element, or parts thereof, in cells of different individuals of a species.

The unpaired chromosomes, as is seen in the X of males, or in the autosomes of young larvae, are segmental in character. In some cases we can see the prophase split and presumably all elements have undergone this process. After the union of homologues in old larvae all trace of the single elements is lost but the dual structure (probably really tetrapartite) exhibits the same morphological differentiations as unpaired chromosomes. So far as I have been able to determine, the union of homologues does not involve much if any twisting of the single chromosomes. It is true that one sees some twisting in incompletely synapsed chromosomes but this has been considered as probably due to the technique employed. In triploid larvae, the three homologues unite in the same intimate way as in diploid cells, but the morphological differentiations are somewhat less pronounced, due perhaps to the thickness of the tripartite element. If one of the chromosomes carries an inversion, such as the delta 49 X, it has been observed that all three elements unite, beginning at the point of spindle fiber attachment and continuing up the point of inversion, and from this point the aberrant element remains single while the other two homologues are paired in the usual way.

All these facts point to the particulate and very specific attraction between the parts of homologous chromosomes. Presumably, the same force operating in salivary gland cells is also responsible for the union of homologues in meiosis, and while we cannot see the actual segregation of normal and aberrant elements in the salivary gland, we can at least determine

how the chromosomes synapse and from this infer, with greater probability, how the same elements would behave in meiosis.

It is interesting to note that the union of homologues takes place in the same intimate way in both male and female larvae. This fact may be interpreted as supporting the conclusion of LEAGUE (1929) regarding the normality of the meiotic phenomena in spermatogenesis and casts doubt on those studies in which the authors attempt to show that no synapsis occurs in the male.

Why homologous chromosomes should unite in somatic tissue is unknown. It is conceivable that it may have an adaptive value, for if the genes are little chemical factories it might be better to have the same type of centers acting under one roof, so to speak, rather than have them scattered. No doubt a good deal of light will be shed on this whole subject through a study of the ontogenetic history of these chromosomes, and it is best, perhaps, not to attempt to interpret the imperfectly known facts at present.

The segments of the X chromosome have definite proportional lengths and each shows a characteristic and often a very striking pattern of lines and bands. So far, I have noted no change in the pattern when the normal position of the segment is changed, as in some one of the various types of dislocations. There are three segments of the X, however, which may show one of two patterns, these are the lozenge, the furrowed and the right hand terminal segments. Apparently, the source of this variation is due to the way the lines are distributed on a segment (and not to quantitative differences of the lines) so that in one case there may be a crowding towards one end forming a conspicuous band, while in another larvae, the lines are more scattered. But a given type of pattern is often (always?) constant for the individual and in the end may have a genetic basis. This is one of the many questions awaiting investigation.

It is needless to say that the ease with which any given chromosome or part can be identified and followed depends on the distinctive nature of its pattern. In this respect the present study has been favored because the X is highly differentiated morphologically. But from almost daily contact and with some detailed study of the autosomes, I feel sure that they will be as easily mapped as the X. If we can thus analyze the chromosomes of a species qualitatively, and follow the fate of a segment in different individuals of a species, there is no obvious reason why the same method of study may not be applied to individuals of related species and, ultimately, perhaps, to widely divergent forms. At the present time, Mr. BREWSTER, one of my students, is making a comparative study of the chromosomes of a number of species of Drosophila. In *D. similans* the topographical features of the chromosomes appear to be identical with those of *D.*

melanogaster, so far as these have been worked out. The writer has been able to identify all of the outstanding landmarks of the X in *D. similans*, in Mr. BREWSTER'S preparations and the left arm of the second is similar in both forms. These initial observations give great promise for the value of this method, in comparative chromosome studies.

It will not be necessary to discuss, at length, the new type of chromosome map herewith presented, nor to point out its obvious practical applications. In contrast to the first cytological maps, such as those of DOBZHANSKY or of the writer, which were based on metaphase plates and were purely quantitative and hence of limited genetic value, the new picture of the X is essentially qualitative. By a study of chromosomal rearrangements of known genetic make-up it has been possible to show that certain gene loci must lie somewhere in a definite segment. With this knowledge, we can study new chromosome rearrangements and determine cytologically what has happened to a certain segment and its contained gene loci. Naturally, as we determine the morphological position of more genes the value of the new map will increase and the day may not be far distant when Drosophila geneticists, interested in chromosomal aberrations, may study the genetics of these breaks by a simple cytological examination of the material. Already, at this laboratory, we are using this method to check up translocations between the X and the fourth chromosome. Once such a translocation is isolated by my colleagues, it is examined cytologically and the point or points of breakage determined with regard to the 21 known gene loci. In this way, in usually half an hour or so, we can determine cytologically what it might take a month or two to find out by the genetic method and, perhaps what is more to the point, we can quickly select breaks which cover a region in which we are especially interested. Maps of the second and third chromosomes are now being prepared.

As already noted, except for the left-hand end of the X, the morphological positions of gene loci, in general, seem to correspond to the crossover values of the genetic map. It should be realized, however, that in most instances we have only been able to place genes within general limits of a segment or more, and that we know nothing, at present, regarding the local distribution within these limits. It is hoped that the studies of Mr. MACKENSEN will throw light upon this subject.

From a theoretical standpoint, the outstanding feature of the present study is the conclusion that neither the inert region of the X, nor of the Y chromosome, except as noted, show as recognizable structures in salivary gland cells. Here we will consider the evidence upon which these conclusions are based.

In metaphase plates of oogonial or of nerve cells, the X chromosome is

considerably longer, on the average, than either arm of the second or third chromosome. In salivary gland cells the arms of these V-shaped autosomes show as separate elements so we might expect the X to be a great deal longer than any other body in the nucleus. This is not the case, however. On the contrary, measurements of a few especially favorable cells showed that the X was materially shorter than either arms of the second or third chromosome. This was the first hint I had that, as compared with oogonial metaphase plates, the X of the salivary gland cells was shorter than we should expect.

The second line of evidence was given by Mr. STONE's "forked-Bar" break (X-IX-4). In metaphase plates, the break separates the X into two pieces of about the same volume. In salivary gland cells, however, the gray segment carrying forked is roughly four times the length and volume of the XR piece which carries Bar. From this we see that the XR element is only one-fourth the size we would expect it to be, judging from oogonial metaphase plates. Or, in other words, about three-eighths of the X chromosome is missing in salivary glands. On the other hand, the size of the forked (XL) segment which is roughly 59 units (in a total of 75.5) from the left-hand end, corresponds well with the crossover value of forked (62), and likewise, the remaining 13.5 units of the crossover map fits well with the 16.5 morphological units of the Bar or XR element. How can the small size of the XR piece be explained? There are two rather obvious possibilities: (1) That the size of the metaphase chromosome is determined by the distribution of the chromatic material and, since the right-hand end of the X is heavily chromatic, as compared to the left-hand end, this causes the Bar segment to appear unduly large in metaphase plates. (2) That the inert region of the X is not represented at all or only in part, in the active salivary gland. There are other possible explanations which we need not consider here. A consideration of the two possibilities just outlined led to a study of deletions, which at metaphase are very large, due to the presence of inert material, but which genetically carry only a small fraction of the genetic map. Deletion 14 was the only stock available for study, but it has proved critical for the questions before us. Genetically, deletion 14 carries less than one crossover unit from the left-hand end, and at the right end only the normal allele of bobbed is known to be present. In metaphase plates of oogonia, however, this deleted X is roughly between a third and a fourth of a normal X in volume. The inert region is what gives the deleted X its large size. In salivary glands, deleted X 14 proved to be very small, altogether not much more than a twentieth of the normal X. As a study of the morphology showed, this size is due almost wholly to the contribution made from the left end, which, of course, is easily recognized. The inert region and the area carrying the normal allele of bobbed consists

of a small nipple-like projection. This makes it abundantly clear that the inert material is not represented, to any appreciable extent, at least, in the salivary gland cells.

The above conclusion regarding the absence of the inert area from the X of salivary glands is in agreement with the finding regarding the Y chromosome, the two lines of evidence giving support to the theory that a part of the X chromosome is homologous to the Y, as WILSON postulated long ago (1911).

The evidence concerning the Y chromosome may be summarized as follows: First, both male and female larvae show the same number of chromosomes, the only difference being that the X of the male is much narrower and perhaps because of this, much less deeply staining than the X of females. From this initial observation it was clear that the Y certainly is not represented by a separate recognizable element. Either it is synapsed with some chromosome, presumably the X, or else it is not present in the salivary glands of males or is represented only by a small unrecognized piece (or pieces) in the nucleus. If the X and Y are synapsed, we should expect that the latter would be very narrow and non-chromatic since the X of males is much thinner and less deeply staining than the paired X's of females.

The first test made for the Y was a study of delta 49 males. The delta 49 X carries an inversion, the morphological position of which we have described in the foregoing pages. When females heterozygous for a normal and a delta 49 X are studied, we get typical inversion figures (see figure 10). If now the Y is synapsed with the X in the region of the inversion, a study of delta 49 males should reveal it. However, although the inverted region was easily identified in the male, the outline of the delta 49 X was perfectly regular at these points, and there was no suggestion of the presence of an achromatic element. This made it clear that as far to the left as furrowed (43.4) there was no trace of the Y chromosome.

To study the right-hand end of the X, males carrying both components of the "forked-Bar" translocation were examined. If the Y is synapsed with the X in the region of Bar (62.2) and to the right of this locus, it should be associated with the XR element. In general, the evidence was negative, that is, no trace of a Y was seen, but in one case I found what I have interpreted as the Y chromosome. This is shown in figure 28. Here the XR element is represented and its right-hand end tip seems to be made up of two components. The normal XR is below and lying on top of this, but distinct from it, except at the point of spindle fiber attachment, is a second structure which has four bands which are apposed to similar bands on the last segment of the XR piece. The outline of the second element is extremely faint and is soon lost in the general coagulum of the nucleus. This

faint element is, I believe, a part of the Y chromosome which, for some reason, has not completely united with the X, and as a result, can be seen. There is some additional evidence which gives strength to this conclusion. That is, it has been shown that the normal allele of bobbed must lie on or about one of the bands adjacent to the spindle-fiber area of the X, and, genetically we know that bobbed is carried by the Y, and this is just the area shown by the Y in figure 28.

Observations similar to those made on the forked-Bar break have been made on a number of the aberrations in which the XR piece was short and with the same result, that the Y could not be found, in a recognizable form. It is safe to conclude therefore that the Y chromosome does not show up in salivary gland cells, like the other chromosomes, and except for the part which has been found in association with the terminal segment of the X and possibly some other fragments not yet identified, it is not present in a recognizable form. In this respect, the Y and the inert region of the X are similar in behavior and thus we have additional support for the theory (discussed at length by MULLER and PAINTER 1932) that the inert region of the X and the Y chromosome are, or were originally, in part at least, homologous.

There are two possible ways of accounting for the behavior of the inert area of the X and of the greater part of the Y chromosome. The first is, that, being genetically inert and hence of no use in ontogeny, the non-active regions have been discarded by diminution or some similar process, as development goes forward. The other explanation is that in the highly specialized salivary gland cells, only the active chromatin of the nucleus, or better, the active genetic material and its associated chromatin, appear in the nucleus as the annulate-like bodies we call chromosomes, and the inert material retains an uncondensed form and contributes, perhaps, to the chromatic coagulum, or reticulum found in all nuclei.[2]

[2] Since this article has been in press an extremely important and interesting paper has appeared by HEITZ (1933) dealing with somatic heteropycnosis in *D. melanogaster* and other Diptera. For a number of years HEITZ has been studying heteropycnosis in plants. He finds that definite sections of chromosomes tend to remain condensed in late telophase and early prophase forming "chromocenters." These chromocenters are made up of "heterochromatin" which HEITZ (1932) thinks is genetically passive or inactive material. The active genetic material, which is called "euchromatin" becomes diffuse at the interphase. With these concepts before him, HEITZ has studied the somatic chromosomes of a number of species of Drosophila, more especially *D. melanogaster*. In the latter species he finds that about half of the X chromosome is heteropycnotic in the interphase, and ties in this observation with the work of Dr. MULLER and myself on the inactive region of the X. In addition, HEITZ shows that both the second and third chromosomes have inactive material in the region of the spindle fiber.

In the salivary gland of *D. melanogaster* the inactive regions of the X and the large autosomes fuse to form what HEITZ calls "sammelchromocentren" or a compound chromocenter which is obviously the same structure which I have described as the "chromatic coagulum." Since this paper was sent to press I have devoted a great deal of time to a study of this compound chromo-

At the present time, the evidence before us does not allow us to decide between these two explanations. It is known, of course, that in Miastor there is an elimination of chromatin, from some somatic cells at least, in development, and while, so far as I know, this process has not been described for any of the Drosophila species it may well occur here. Were the inert chromatin of the X or the Y physically eliminated from the nuclei of the salivary glands, then we can understand why the only trace of the Y found is the short active area which carries the gene for bobbed (and possibly other small areas as yet unidentified) and why the X is relatively too short. At the same time we would have a simple genetic explanation to account for the enigmatic process of diminution, which has been described here and there in the ontogenetic history of widely separated forms. This problem is one of the most interesting which have resulted from this type of cytogenetic study and its solution obviously lies in a study of somatic mitoses of various types of tissue in developing larvae. If metaphase plates of very young salivary gland cells show the X and Y as we see them in germinal tissue, then the alternate view, that the inert areas are present in some unrecognized form, would be indicated. The latter view finds some support in the genetic evidence we have for the X. The crossover map of the X and the morphological picture we get from a study of salivary glands, correspond very closely. Whatever the nature or function of the inert region may be, the sex chromosome at meiosis behaves as if it did not exist, so far as crossing over is concerned, a fact which could mean that at this critical period the inert region was not an organic part of the X chromosome.

From the present study, we may conclude that, in metaphase plates about three-eighths of the X chromosome is made up of inert material.

In the foregoing discussion, I have limited myself to only a few of the many points which will have suggested themselves to the reader. Foremost stand questions about the intimate structure of chromosomes, whether the chromatic bands lie on or within the matrix? What part carries the genes? What is the relation between the genes and the chromatic substance? and a host of similar questions. From the first, such questions have dominated

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center and a full discussion of my findings are given in a paper now in press (PAINTER and STONE). From a study of breaks in the second and third chromosome I am convinced that HEITZ is correct in his conclusion that both these autosomes have inert areas in the region of the spindle fiber. On the other hand, in my opinion, the total volume of the chromatic coagulum or chromocenter in salivary glands is not large enough to account for all of the inactive material of oogonial or nerve cell metaphases. It is possible that the active euchromatin, representing the banded areas of all the chromosomes, is more greatly hypertrophied than the inert material, or that some of the inert chromatin is in solution and hence not easily detected by the technique used, or that some of it has been eliminated by diminution. Up to the present moment I have no critical evidence to decide between the various possibilities.

our interest in these studies, but as tempting as it may be to speculate upon them, at present it seems unprofitable to consider these points until we know more about the genesis and ontogenetic history of these peculiar chromosomes. This latter field has been explored somewhat, in other dipteran forms, by ALVERDES (1912) and by TANZER (1922). While these several accounts differ in details, it would appear that, in salivary glands, the chromosomes are differentiated beyond the point reached by cells which will subsequently undergo division. What is needed now is a very thorough study of this whole period, in the larva of *D. melanogaster*, or some closely related form, and with this background we can consider the questions of chromosome structure, with more assurance of being able to make definite progress in the general field. This line of work is being pushed at present here in Austin.

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